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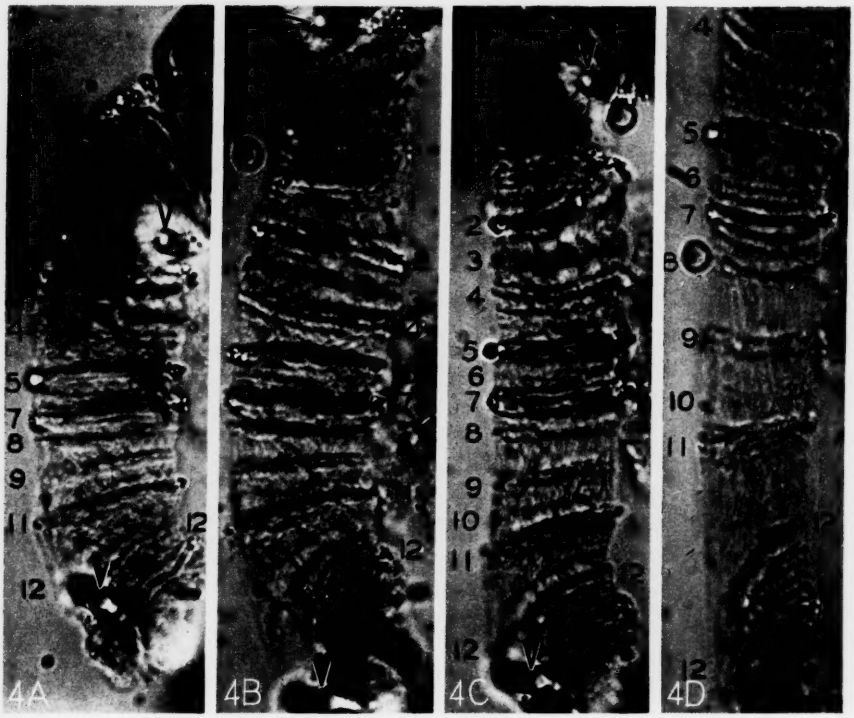

CHROMOSOMES STRETCH LIKE RUBBER BANDS

(See Page 3)

Twins in the Study of Child Behavior

Social Behavior of Inbred Mice

A Spontaneous Amphidiploid

A Pedigree of the Genetic "Hot-Foot"

The White Belly Gene in the House Mouse

VOLUME 33 JANUARY, 1942 NUMBER 1

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CONTENTS

MICROMANIPULATION OF SALIVARY GLAND CHROMOSOMES
By John B. Buck 3

GENETIC DIFFERENCES IN THE SOCIAL BEHAVIOR OF INBRED STRAINS OF MICE
By J. P. Scott 11

A NEW PEDIGREE OF RECURRENT BULLOUS ERUPTION OF THE FEET
By J. B. S. Haldane and Rosamunde Poole 17

THE INHERITANCE OF "WHITE-BELLY" IN THE HOUSE MOUSE
By Nathan Kaliss 21

A NATURAL AMPHIDIPLOID FROM AN ONION SPECIES HYBRID
By Henry A. Jones and Alfred E. Clarke 25

Notes and Reviews

BLOCK THAT STENOSIS 10

"OFF THE RECORD" TWINS AND MICE 15

TWINS AND THE STUDY OF DELINQUENCY (*Rev. of* ROSANOFF, A. A., L. M. HANDY AND I. R. PLESSET, *The Etiology of Child Behavior Difficulties*) 18

ARE INSECTS PEOPLE? (*Rev. of* LUTZ, FRANK E., *A Lot of Insects*) 23

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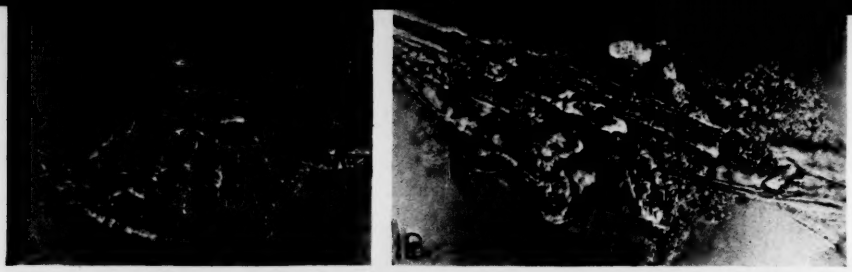
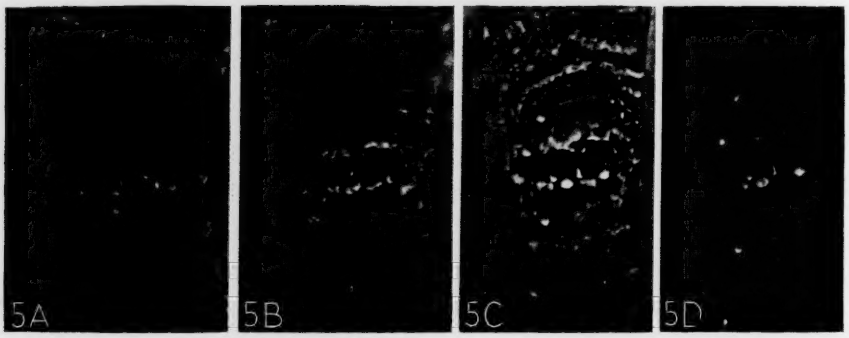
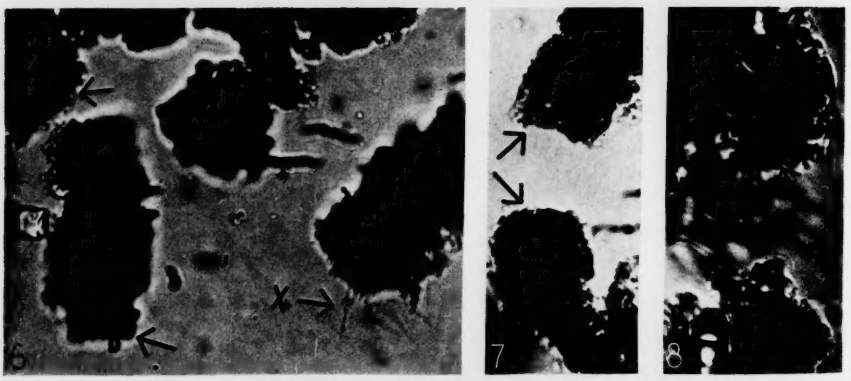
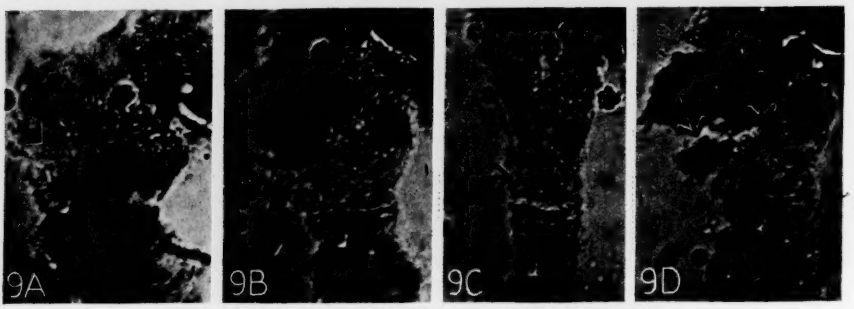








EFFECTS OF STRETCHING ON CHROMOSOMES

Figures 1, 5, 6, 7, 8 and 9

Figure 1. Entire untreated nucleus isolated in Ringer's solution. "C" shows cytoplasm adhering to nuclear membrane. 1*A*, slightly stretched; 1*B*, more strongly stretched. Note viscosity and stickiness of chromosomes. The apparent "pellicle" at "X" in 1*B* will be discussed in another paper. × 450. In this and subsequent figures, "V" shows position of needle point. All figures, except number 1, 2 and 13 are from chromosomes isolated by the osmic vapor treatment.

Figure 5. "Nucleolar" band region of a chromosome under different degrees of tension. 5*A*, unstretched; 5*B*, 40%; 5*C*,130%; 5*D*, 210%. × 850.

Figures 6. 7 and 8. Square ends of chromosomes broken by excess stretching. Arrows indicate broken ends. "X" shows fibers where chromosome "frayed" in breaking. × 850.

Figure 9. Reversible transformation of alveolar structure into striations. 9*A*, unstretched, arrow indicating staggered rows of vesicles forming a honeycomb structure; 9*B*, 70% stretch, arrow indicating appearance of diagonal striations; 9*C*, 240% stretch, very pronounced striations; 9*D*, relaxed, honeycomb structure again. × 850.

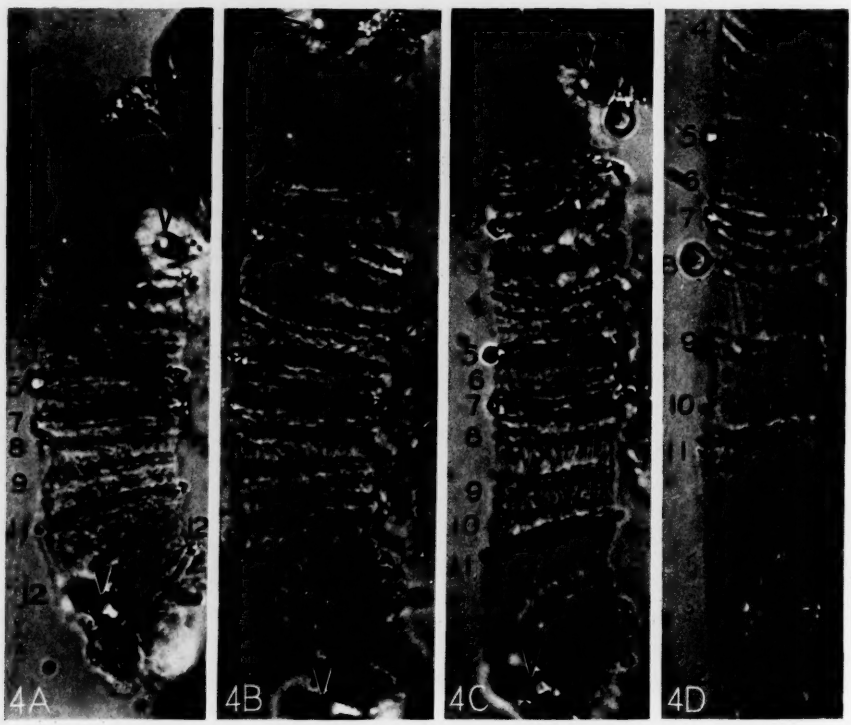
MICROMANIPULATION OF SALIVARY GLAND CHROMOSOMES

JOHN B. BUCK
*University of Rochester**

THE physical properties of chromosomes were first studied directly by microdissection by Chambers and Sands[12] in *Tradescantia* and by Chambers[10, 11] in *Dissosteira*. Working on fresh cells, these investigators found that the prophase chromosomes are cylinders with a homogeneous elastic core and a cortex containing granules which become more widely separated when the chromosome is stretched. They reported also that the metaphase chromosomes are optically homogeneous elastic rods. Wada,[32] investigating *Tradescantia* nuclei in 26 per cent sucrose and dilute K and Ca solutions, found that the coiled chromonema can be straightened out by stretching. His Figure 7, however, indicates that the chromonema is not elastic.

With the reinterpretation in 1933 (by Heitz and Bauer,[19] and Painter[25]) of the structures in salivary gland nuclei of *Diptera*, chromosomes became available which offered new promise for micromanipulation. Vonwiller and Audova,[31] working on *Chironomus sp.* in blood and in 0.6 per cent NaCl solution, found that these giant chromosomes are of high consistency, pressure or tension on one part being transmitted to all other parts; that they are elastic and can be stretched to "many" times their original length and then spring back (but not to their original length) when the tension is released; that the cross-banding is maintained if the stretching is not too great; and that in breakage the break comes at the boundary between single bands. Barigozzi,[3] working on *C. thummi* in amphibian Ringer, observed that the gelatinous refractile chromosomes adhere to the needles; that they return to their normal size and appearance after being stretched to double their length; and that under repeated stretchings they first transform into bundles of parallel longitudinal filaments, and then "dissolve" completely. Stefanelli,[30] working on an unidentified chironomid in plasma and in Ringer's solutions, maintained that the chromosomes are not sticky while in the nucleus (since they do not stick to each other when the nucleus is deformed) but only become so when the nuclear membrane is ruptured; that the viscosity of the chromosomes defeats every effort to separate the homologs (see similar conclusion of the writer[7]); that under tension ("1/3 elongation") the cross-bands become less distinct, but reappear when the chromosome is allowed to contract to its original length; that a stretch to twice the original length of the chromosome exceeds its elastic limit and produces irreversible deformation and longitudinal striation; that the clear inter-band regions resist deformation less than the heavily banded areas; and that the chromosomes solate entirely and disperse in highly hydrated nuclei. Pfeiffer,[27, 28] working on *Chironomus* in body fluid, 0.6 per cent NaCl and paraffin oil, maintained that the chromosomes show considerable rigidity, that they withstand a several-fold elongation without breaking, although they do not return to their original length; and that the chromo-

*This work was done at the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland, and at the Marine Biological Laboratory, Woods Hole, Mass., as part of a series of chromosome studies made in the laboratory of Dr. C. W. Metz with the support of the Rockefeller Foundation. The writer is much indebted to Dr. Metz for advice and encouragement. The Carnegie Institution generously bore the c st of the plates, which were prepared with the aid of Mr. Chester Reather of that institution and Mr. Ray Maas and Mrs. H. D. Stalker of the University of Rochester. The assistance of Rochester colleagues and of Professors Chambers and Mast with the manuscript is acknowledged with pleasure.



BANDING IN STRETCHED CHROMOSOMES

Figures 2, 4 and 13

Figure 2. Chromosomes from fresh nucleus isolated after treatment with albumin. Arrows indicate regions of recognizable cross-banding. Note extreme plasticity of chromosomes. × 680.

Figure 4. Region of another chromosome similar to that in Figure 3. 4*A*, unstretched; 4*B*, 16%; 4*C*, 50%; 4*D*, 165%. × 850.

Figure 13. Chromosomes isolated from larva killed with ether. Note absence of bands, homogeneous structure, and inelasticity. × 700.

somes decrease in diameter when stretched (observed also by Barigozzi). He also noted, as had other workers, that most of the elongation occurs in the interband regions, and he observed that these regions (which in relaxed chromosomes are isotropic, in contrast to the bands, which are negatively birefringent) become increasingly birefringent in polarized light as the chromosome is stretched. This change he associated with a transformation from a folded α-polypeptide chain to an extended β-chain, according to Astbury's[1] interpretation of the structure of fibrous proteins. Glancy[18] extended the observations of Vonwiller and Audova and Stefanelli on the nuclear sap of *Chironomus* nuclei.

Duryee[14-17] investigated "lampbrush" chromosomes in the germinal vesicles of immature amphibian eggs in Ca-free Ringer. Among other effects, he observed that the chromosomes withstand an average stretch of 450 per cent before breaking; that a single chromosome was stretched to double its length 125 times without "altering its relaxed appearance"; and that when 0.001 M. $CaCl_2$ is added, the chromosomes become more brittle.

Materials and Methods

The work reported here was done primarily on the salivary gland chromosomes of mature *Chironomus plumosus* larvae. These chromosomes are solid banded cylinders sometimes as large as $150 \times 20 \times 20$ microns. It was early found that dissection of "normal" or "living" chromosomes from fresh glands in blood or salt solution is infeasible because dissection of the cells causes an immediate, marked and irreversible condensation and shrinkage of the chromosomes. Even these shrunken chromosomes, although they may be somewhat elastic, are not suitable for dissection because both they and the cytoplasm are extremely sticky and plastic. These characteristics are well shown, for example, by the chromosomes in nuclei dissected out of fresh glands in Ringer's solution (Figure 1) and in nuclei isolated by soaking glands in 0.25 per cent egg albumin for two to three hours[22] (Figure 2). Both treatments appear to leave the chromosomes in a quasi-normal state.

It was accordingly necessary to subject the chromosomes to some kind of pre-treatment to render them suitable for micromanipulation, even though they could then no longer be considered normal.

The main method used was as follows: The glands were dissected out and put into a hanging drop of isotonic Ringer's solution* on a coverslip, which was then sealed with Mayer's albumin over a depression slide containing a drop or two of 2 per cent osmic acid solution and left for 18 to 24 hours at 5°C. This rendered the cytoplasm brittle enough so that nuclei could be dissected out and opened to obtain the chromosomes.† The dissection was made by hand in distilled water with glass needles. No stain was used.

Chromosomes thus isolated were put in a hanging drop of distilled water in a moist chamber, and manipulated with a standard Chambers micromanipulator. The magnitude of the stretch to which these chromosomes were subjected was ascertained by measuring the distance between two selected bands, with each successive increase in tension. The "stretch" or elongation is expressed in percentage of the original length of the unstretched chromosome (e.g., "200 per cent stretch" means that the chromosome elongated until it was thrice its original length).

The conclusions are based on 56 manipulations (each on a different chro-

*Composition: 5.55 g. NaCl, 0.22 g. KCl and 0.44 g. $CcCl_2$ per liter. This was judged isotonic because nuclei in glands transferred to it from body fluid did not change in volume.

†The chemistry of this action of osmic vapor (and of formalin) will be considered in detail in another paper,[8] so it need only be said here that the effect is mild, probably not even involving coagulation of proteins. Cytologically, the chromosomes resemble those in the living animal[6] very closely in visible structure and in size (see aso Doyle and Metz[13]) and remain flexible and elastic. The only perceptible effect is a reduction in surface stickiness.

mosome) of which 29 were photographed stage-by-stage at an initial magnification of 268×, using a 3.5 mm. Zeiss apochromatic oil immersion objective and Zeiss "Phoku" camera. All figures are unretouched.

Results

Figures 3 and 4 show typical results of simple stretching experiments. Each series of photographs shows the same region of a single chromosome under different degrees of tension, the numbers indicating the successive positions of particular bands. From these experiments (and others to be cited later) we can draw the following conclusions concerning the visible structure and physical properties of these chromosomes:

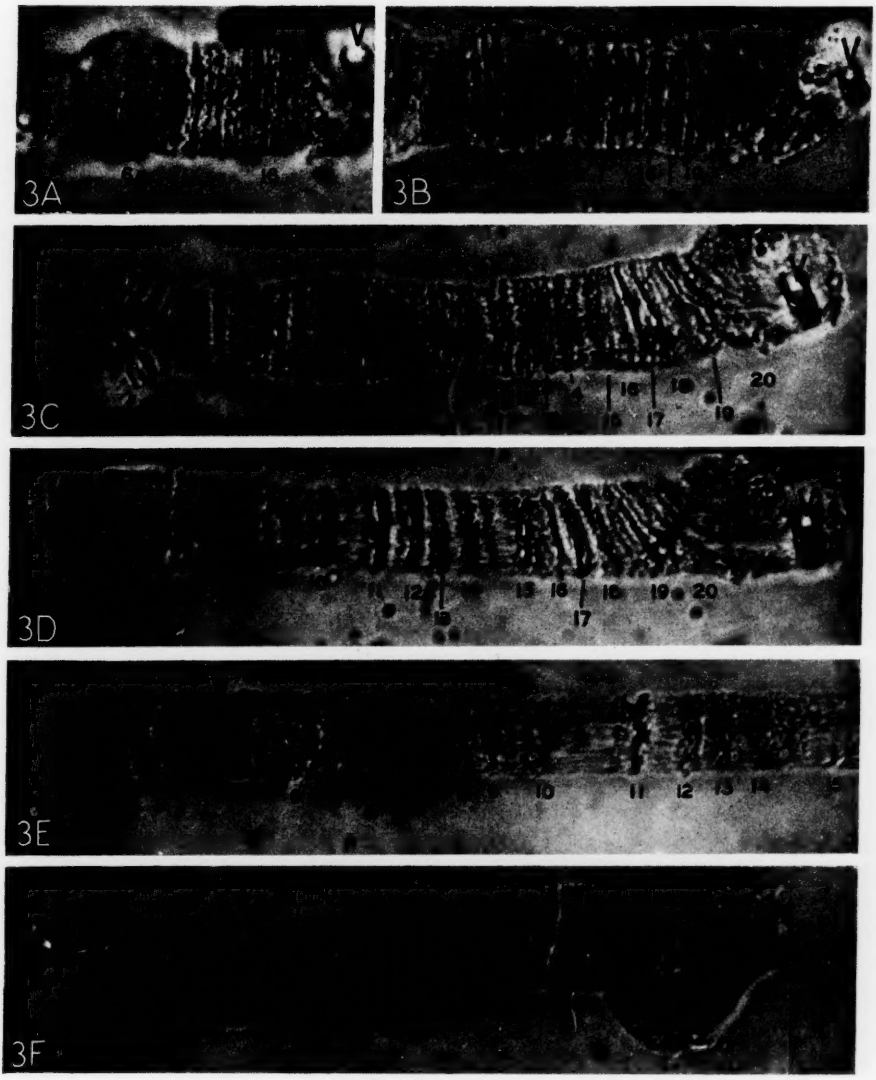
(a) Most of the stretch comes in the interband regions, whereas the bands themselves seem either not to stretch or at most to demonstrate their compound nature by separating slightly into several narrower bands. This conclusion is illustrated, for example, by the interband regions 4-5 and 10-11 in Figures 3*B*, 3*C*, 3*D* and 3*E* (respectively 20, 80, 175 and 300 per cent stretch); by the interband regions 8-9 and 11-12 in Figures 4*A*, 4*C* and 4*D* (0, 50 and 165 per cent stretch); by the bands 17 and 18 in Figure 3 and bands 10 and 11 in Figure 4 and in the peculiar "nucleolar" band in Figure 5. The regions of heavy bands stretch less than the regions of delicate banding as seen for example in comparing the regions 12-13 and 13-14 in Figure 3. It may be recalled that the banded regions have been shown by Caspersson,[9] by Mazia and Jaeger[21] and by Mazia[20] to be rich in nucleic acid.

The converse experiment, the longitudinal compression of chromosomes, or the attempt to force the bands closer together, does not work readily, because the chromosomes buckle without any shortening.

(b) The chromosomes seem to increase in volume during stretching. This can be only a tentative conclusion because of the roughness of the measurements, but considering the chromosomes as cylinders of circular cross-section, calculations indicate that the region 8-14 increases about 60 per cent in volume between Figures 3*B* and 3*E*, and the region 5-11 increases about 45 per cent between Figures 4*A* and 4*D*. In other words, diameter seems not to have decreased as much as would be expected if volume had remained constant.

(c) When chromosomes are stretched, longitudinal striations not previously visible usually appear in the interband regions. This is seen, for example, in the regions 10-11, 11-12, 13-14, 4-5 and 7-8 of Figure 3, and in the regions 8-9, 9-10 and 11-12 in Figure 4, and many other examples could be adduced. Except under conditions of extreme stretch these striations tend to run diagonally or crisscross at every focal level rather than parallel to the long axis of the chromosome. If the elongation is not extreme, these striations disappear when the chromosome is allowed to relax, but with extreme elongation the striations remain, and the chromosome appears to be altered permanently, as shown also by its reduced elasticity. If these striations were the actual genonemata as claimed by some investigators, they ought to be even thicker and more pronounced in the unstretched than in the stretched chromosome. Since they are not it seems more reasonable to regard them as stress-line artifacts, as maintained by Metz.[23,24] It should be mentioned, however, that similar diagonal striations are also sometimes visible in slightly shrunken chromosomes within intact nuclei both in fresh and fixed material in both *Chironomus* and *Sciara*.

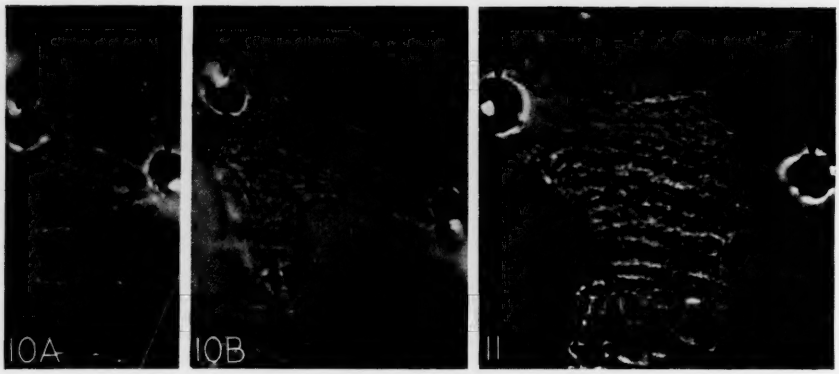
(d) The chromosomes (at least after osmic vapor treatment) are very elastic. In Figure 3, the chromosome returned to a length only 40 per cent greater than the original after having been stretched to over four times its original length. In another example the chromosome contracted to only 27 per cent longer than before stretching after having been stretched about 175 per cent. Just what stretch can be withstood with no loss in elasticity is uncertain but from measurements on 30 chromosomes is estimated to be about 100 per cent. Apparently over 300 per cent stretch can be with-



BAND-SHIFTS UNDER TENSION

Figure 3

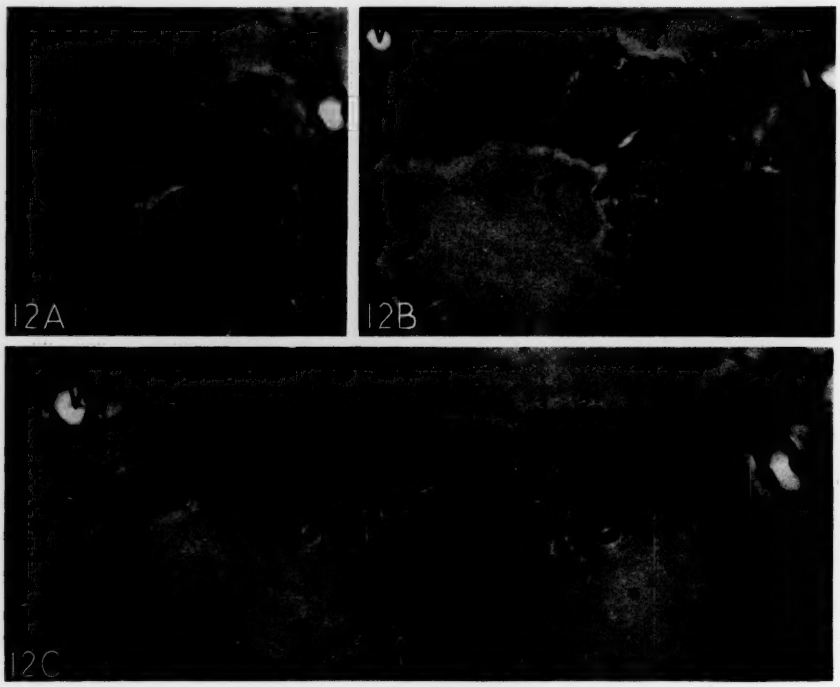
Region of single chromosome, under different degrees of tension. *3A*, unstretched; *3B*, 20% elongation; *3C*, 80%; *3D*, 175%; *3E*, 300%; *3F*, relaxed. Note that most of the elongation is in the interband regions. See also text. Numbers indicate positions of same individual bands in successive figures. × 850.



STRUCTURE UNDER TRANSVERSE STRETCH

Figures 10 and 11

Transverse stretching of chromosomes. Note transverse striations due to tension, and absence of longitudinal fibrous structure. × 850.



HOMOLOGS SOLIDLY JOINED

Figure 12

Attempt at separating somatically synapsed homologs. Chromosome showed natural terminal asynapsis in region 1-4, but very strong tension failed to separate the homologs further or to disclose evidence of doubleness in region of synapsis. × 850.

stood before the chromosome breaks. The fact that the chromosomes return to their original length after being stretched to double that length, suggests, like the work of Pfeiffer, an analogy with the (hypothetical) α-β polypeptide chain interpretation of Astbury, but the stretching experiments are too crude to support any safe conclusion in that direction, particularly in view of our fragmentary knowledge of chromosome chemistry.

(e) When a chromosome is stretched too far it invariably breaks in an interband region, and directly across the diameter of the chromosome. Such square broken ends are shown in Figures 6, 7 and 8. Of particular interest also are the "frayed" ends with threads projecting (Figure 6, x). The material is apparently of sufficient viscosity to be drawn out into strands. Alternatively, of course, they could be regarded as broken chromonemata (*vide infra*).

(f) In Figure 9*A* the arrow indicates a region of an unstretched chromosome where the structure is clearly alveolar or foam like, with parallel staggered transverse rows of tiny droplets forming a honeycomb-like structure. Figure 9*B* shows the same region under 70 per cent stretch; note that parallel diagonal stress lines ("striations") have begun to appear, apparently formed by the alignment and distortion of the walls of the little droplets, some of which are still visible. In Figure 9*C* (240 per cent stretch) the transformation is even more striking. Finally, in Figure 9*D*, the same region is shown again, after the tension has been released, and the alveolar structure is again visible. These observations tend to support the contention of Metz that (at least in *Sciara*) the longitudinal striations are artifacts caused by the apposition of the walls of the alveoli rather than actual continuous physical threads or fibers (chromonemata) as maintained by Bauer[4,5] and Painter.[26]

(g) Further information as to the physical nature of the striations might be furnished by attempts to shred the chromosomes longitudinally. Figures 10 and 11 show the results of several relatively crude essays in this direction, which at least do not disclose the presence of any pronounced longitudinal fibrous structure or of any tendency for the chromosome to split longitudinally.

(h) Another piece of evidence on the longitudinal structure of the chromosome is furnished by experiments on the (somatic) synaptic attraction between homologs. In *Chironomus,* apparently, partial terminal asynapsis of homologs is not infrequent. Such a forked chromosome was manipulated in an attempt to pull the two homologs apart. As seen in Figure 12, the two homologs resisted a very strong lateral pull without showing the least tendency to separate.

(i) Chromosomes from larvae killed by ether are optically homogeneous, and so plastic that they stretch indefinitely (Figure 13) without exhibiting the slightest elasticity.* Much further work is required before this result can be understood, particularly since it is not even certain that ether reached the chromosomes, but since the bands disappear it looks as if ether attacks the bonds joining nucleic acid to the protein framework. Morever, pursuing the tenuous analogy between chromosomes and fibrous proteins, the ether may be considered to have affected somehow the bonds joining the polypeptide chains together, thus allowing "intermolecular slipping."[2] This suggestion of a bonding rôle of lipoid in the chromosome fabric is perhaps supported by the observations of Shinke and Shigenaga[29] of dissolution of chromosomes in certain fat solvents.

(j) Subsidiary observations of some interest are: Chromosomes isolated from nuclei treated with formalin (46 hours in 10 per cent at 8°C.) and with acetic acid (an hour or so in 45 per cent) are quite elastic and should repay further study. Several chromosomes from *Sciara coprophila,* isolated by the osmic vapor technique, were manipulated and found to be very similar to those in *Chironomus.*

Summary

In larval salivary gland chromosomes of *Chironomus plumosus,* isolated by an

*No osmic treatment used here.

osmic vapor technique, the following results were obtained by micro-manipulation:

1. The chromosomes will withstand about 100 per cent elongation without permanent deformation, and about 300 per cent before breaking. Most of the elongation occurs in the inter-band regions. Breakage occurs straight across the chromosome at right angles to the long axis. There are indications of a volume increase during stretch.

2. Under stress, diagonal and criss-cross striations appear in the inter-band regions. In some instances these can be seen to be formed by the apposition of the walls of vesicles from an originally honeycomb-like structure. The striations are regarded as artifacts rather than genonemata.

3. No evidence of any regular longitudinal differentiation was obtained in attempts to "shred" the chromosomes and to separate the synapsed homologs.

4. After ether treatment, chromosomes are plastic, suggesting a possible rôle of lipoid in bonding the fabric.

5. The elasticity, and certain other properties of salivary gland chromosomes suggest a tentative chemical comparison with those of fibrous proteins.

6. A review of micromanipulation work on chromosomes is included.

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Block That Stenosis!

Dr. Irving Cutter, in his syndicated medical column, "How To Keep Well," tells the Jan. 6, 1942, world how easy it is to prevent nervous stomach genes from putting their kibosh on the next generation:

An Unwanted Legacy

R. M. writes: Can a nervous stomach be inherited?

Reply

Yes, decidedly. The practice of self-control may preclude the possibility that you will pass the tendency on to your children.

GENETIC DIFFERENCES IN THE SOCIAL BEHAVIOR OF INBRED STRAINS OF MICE

J. P. Scott
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IT is almost a truism that our greatest practical problems—war and peace, crime and punishment, work and relief—are found in the field of sociology. As attempts to solve them get under way it is equally obvious that they are partially included in most other fields of science. The material below indicates that interesting possibilities may arise from an approach to social difficulties by way of genetics.

Plans for practical remedies usually bring up two basic theoretical questions: 1) What causes individual differences in social behavior? and 2) What causes the social behavior of an individual to change? The answer to the first question limits the amount of division of labor and hence of organization in any society, and that of the second determines its capability for adjustment.

Broad and general answers can be given to both queries. Differences between individuals may be caused by differences in the present or preceding environment, in age and sex, and in physiological conditions related to all other causes, and in heredity. On the other hand, changes in the behavior of an individual may be produced by changes in the social, biological and physical environments, changes in age, and by related changes in physiological conditions.

The first problem is more fundamental, and an opportunity to test one of its causes, heredity, was seen in the large number of inbred stocks of house mice kept at the Roscoe B. Jackson Laboratory* at Bar Harbor. The result was not only confirmation of the importance of this factor, but it yielded fertile suggestions as to methods for experiment with other factors.

The general technique of the project was simple. The nature of inbred stocks has been ably discussed by W. L. Russell[1]: the primary effect of long-continued inbreeding is to make most of a particular combination of genes homozygous, but the action of one such combination may be much more easily modified by minor fluctuations of environment than that of another, leading to a larger amount of variability in the outward character of the stock. Since all of the stocks tested had been inbred without selection for at least ten generations of brother-sister matings, each one could be assumed to be very nearly pure-breeding and to carry a combination of genes more or less different from the rest. Thus it was expected, if heredity was a cause of social behavior, that the stocks would differ from each other both on the average and in the amount of variability, even when kept in the same environment.

Observations were planned so that the results would be as simple and clear-cut as possible. One of the chief difficulties of behavior work is the time it takes, and a deliberate attempt was made to select certain stocks which showed no overlap and hence whose average differences could be verified in a short series of comparisons. No effort was made to study variability, which has to be estimated from large numbers.

For similar reasons, a trait was used which could be expected to show little variation under constant environmental conditions. The most important kinds of social behavior found in mice are sexual activity, maternal care, aggregation in the nests, and fighting. The first two are always changing because of the endocrine cycles of the females, and the third is partly dependent upon the other

*The author wishes to thank the staff members, without whose aid and hospitality this work could not have been done.

three. Fighting between adult males was finally chosen as the easiest trait with which to work.

It was thought that the biggest chance for a mistake would lie in some unrecognized environmental difference between the stocks, and a careful search was made for this. However, all mice were cared for under the systematic method described by Bittner[1], and only two possible differences were found. During the months when the experiments were made (July and August, 1940), the temperature in the building, though fairly even, was not the same in all parts, and the stocks were handled and cared for by several workers.

There appeared to be no chance that the mice could learn different sorts of behavior from their respective parents. According to the system of inbreeding used at the laboratory, a male and from one to four litter-mate females were kept in each breeding cage. When pregnant, a female was removed to a separate box, and the young were separated from her before maturity. Since the females were never seen fighting and the males do not fight until sexually mature, it was possible to watch males which had never seen a battle enter upon their first conflicts.

By a lucky chance, each mouse could be matched against one from a group having similar heredity and experience. A long series of Bagg albino males, kept five in a box since weaning, was available, and one of these was placed in the home cage of each male to be tested. A new albino male, at least three months of age, was used for every observation.

In the preliminary tests these Bagg albino males reacted as follows: On being placed in a strange pen they smelled and sniffed first the walls of the cage, and then its occupants (investigating behavior). After five to seven minutes, if not already attacked by a home male, they usually began a series of aggressive nudgings or an outright attack.

In the final series of experiments, done during a period of warm weather in the middle of August, the Bagg albino males were much more lethargic, investigating the pen and its occupants in a very cursory fashion, showed no aggressive behavior and spent a good deal of time spread out flat on the bottom of the cage.

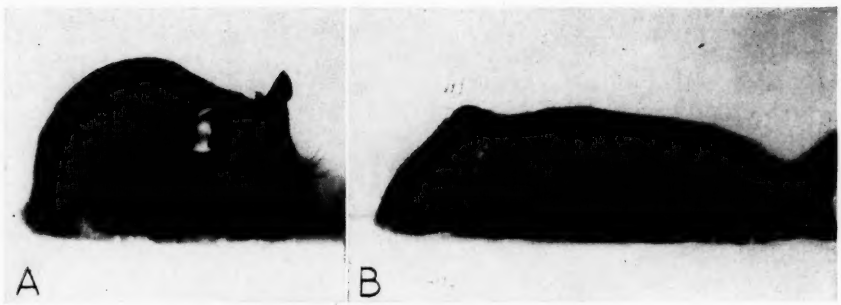
Observations were made in a simple routine. The breeding box was brought down into a laboratory room, the water bottle removed, and a 60 watt student lamp installed about seven inches above the top of the cage. In that way the actions of the mice in the box could be seen and the mice could not easily see the observer. (This also had the effect of raising the temperature inside the box about 4° F. above that of the room, which varied between 68° and 78° F.) If there were females present their number was reduced to one. The Bagg albino male was then introduced and activities noted over a period of ten minutes. As soon as a fight started the strange male was taken out in order to avoid injuries to valuable breeding animals. Thus the data refer only to preliminary fighting behavior and do not include the outcome of the conflict.

Preceding a fight the home males might do any of the things listed in Table I, and these were checked for each two-minute period. In later experiments a set of key numbers was memorized and a record kept for successive activities timed to the minute.

Giving the names under which the stocks are listed at the laboratory, a preliminary survey of the *C57* blacks (subline 10), *C3H* agouti, *C* (Bagg) albino, *dba* dilute brown, c^d extreme dilute, *C57* brown, *C57* leaden and *P* pink-eyed dilute was made during July when the *C* albino males were active and aggressive.

These first results indicated, as was expected, that each stock had individual peculiarities, although there were many cases of overlap. An exception was noted in the *C57* brown and leaden animals which had been derived from mutations of the *C57* black strain. These were very similar in behavior to the *C57* black.

Three strains were selected which seemed to be entirely different from each other. The *C57* blacks (subline 10) always showed immediate interest in the intruding male and made many contacts, at times apparently licking and cleaning



"PACIFIST" AND "AGGRESSOR" STRAINS

Figure 14

A *C57* black male (left) and a *C3H* agouti (right), both about four months of age. The more active attitude and sleeker fur of the *C57* type appears even in photographs. In several important "social" characteristics these two inbred strains differed consistently and widely.

him. There was no evidence of aggressive behavior, although they fought back when attacked. The *A* albino males made very little preliminary contact with the intruders and usually attacked them within a couple of minutes. Finally, the *C3H* agouti males tended to sniff briefly at the intruder and then to keep to the opposite side of the pen for several minutes. During this time the fur fluffed up and the animals breathed in a labored fashion. If the intruder had not attacked in the meantime the *C3H* males eventually started a fight, usually preceded by a series of feints and withdrawals.

Further paired tests (males of the same age, observed within a few minutes of each other) were then made in which these peculiarities were carefully watched for. The following results were obtained when the *C* albino males were passive and non-aggressive, presumably because of hot weather.

In paired comparisons between four *C57* black males and five *C3H* agoutis made on August 17, 18 and 19, 1940, five distinct differences in behavior were recorded in every case:[2]

1. The *C3H* males sniffed lightly at the intruder and then withdrew whereas the *C57* males made close contact.
2. The *C3H* males avoided the intruder for some minutes whereas the *C57* males made many contacts. (Recorded in three times as many two-minute periods.)
3. The *C3H* males showed fluffing of the hair and jerky labored breathing whereas nothing of the sort occurred in the other males.
4. The *C3H* males attacked the intruders and the *C57* males did not.
5. This attack was preceded by feints on the part of the *C3H* males; no such activity was observed in the *C57* males.

Assuring two similar but variable stocks (the most plausible alternate conclusion) each of the above results would be obtained by chance about one time in sixteen, and since the preliminary data were consistent, such a possibility is remote.

However, the new conditions (warmer weather and passive behavior of the *C* albinos) seemed to affect the *A* albinos very markedly. In certain cases behavior was almost identical with that of *C3H* males, and in paired comparisons between six *A* albinos and four *C57* blacks made August 17, 18, and 19, 1940, there was a consistent difference only in the following respect: that the *A* albinos made light and unrepeated contacts with the intruder. In some cases they lay down flat as if suffering from the heat. It was concluded that the stock is probably one in which a large amount of variability is caused by small changes in the environment.

A further series of tests made on August 12-14, 1941, will be of practical interest, as neutral pens have often been used by other observers. This series differed from the others in the following ways: the pen was strange to both ani-

mals, the temperature was consistently kept at 68-70° F., no females were present, and each *C* albino male was used two or three times. Only the first two of these factors appeared to be important, one causing increased investigation of the pen, and the other active aggression by the *C* albinos.

A series of six *C3H* males showed the same sort of behavior as before, except that aggressiveness was delayed, possibly because of their paying more attention to a new pen. In some of them hair fluffing began before they saw the other male; this was apparently caused by handling. The *A* males varied as before, between active aggression and passivity, and some overlapped the *C3H* type.

The new conditions made the greatest change in behavior of the *C57* stock. Instead of nosing the other male they investigated the pen itself, running around from one corner to another in vigorous activity. The albinos made an unusually large number of attacks, which were disregarded unless severe, the attacking male being shaken off or pushed aside. A single exceptional male was found which was aggressive almost from the start and which finally attacked the *C* albino. This male had been alone in its home pen, which might account for its unusual behavior.

With added cases and new conditions there was still no overlap between the *C57* and *C3H* strains, and these two appear to be the most interesting and useful for bio-sociological studies.

It should be emphasized that the differences described occurred in two special environments, whose peculiarities may be summarized as follows:

1. The nature of the wooden pens tended to isolate the mice from outside disturbances, but they were exposed to variations in light.
2. The experiments were done during the summer months in a climate where there was relatively little temperature fluctuation.
3. The pen and contents were changed every few days, and the mice were frequently handled.
4. There were no substantial nests built.
5. The males might occasionally be alone, but had frequent contacts with females. They had no contacts with young mice except by accident, and they had no previous contacts with males except their litter mates before weaning. Thus the fights observed were probably first fights, and the records deal with preliminaries more than with fighting itself.

Under other conditions (constant temperature, isolation, complete darkness except at times of observation, wire cages, nesting material provided, and repeated fights) Fales and Ginsburg[3] at the University of Chicago have found that the *C57* blacks are more successful than the *C3H* in fights which are allowed to go to a finish. It is evident that the pacific attitude of the former stock is not maintained under all conditions.

In any sort of environment so far studied distinct differences between the two strains have been found. This indicates that important variations in the so-

TABLE I. Commonly Observed Activities of Male Mice

Type of Behavior Affecting:	*Pen and Contents*	*Self*	*Other Mice*
Miscellaneous	Eating Digging in shavings		
Investigating	Sniffing pen and feeling with whiskers		Sniffing and nosing
Grooming		Licking fur Scratching	Licking fur
Sexual		Licking genital region	Nosing genital region Mounting
Fighting	Biting on wire	Hair fluffing Jerky, labored breathing	Twitching tail (may be rattled against cage) Biting whiskers Nudging with nose Feint Fighting back after attacked Attack (striking, biting, and wrestling)

cial behavior of mice can be caused by heredity, and this is supported by previous data concerning the photic response in inbred stocks of Drosophila.[4]

One of the most fertile methods in the study of general sociology is the carrying-over of information and techniques between inter-related fields of knowledge which are usually widely separated by tradition and terminology. However, this needs to be done sceptically rather than enthusiastically. While it is true that the same laws of heredity hold for man and mouse, it is also true that inbreeding produces rare combinations of genes including many recessives, and human beings are almost never highly inbred. It is therefore proper to suggest only that human social behavior *may* be affected by heredity.

A more important use of this material is that it offers a chance to duplicate certain simple human situations involving what is ordinarily known as "character." For example, when nest building material of cotton and excelsior is provided, the *C57* strain apparently prefers cotton, the *C3H* excelsior. The *C57* mice keep their pens tidier and build solider nests. All of this seems to be related to certain simple traits. The *C57* is an active type making close contact with its physical and social environment and is slow to fluff the hair, while the *C3H* is relatively inactive, avoids close contacts, and is quick to give the involuntary hair-raising reaction.

Thus it should be possible to watch one type of mouse adjusting to another. Fostering mice into another stock would give a test of early social environment. The easily induced hair-fluffing of the *C3H* strain is a reaction which might lend itself to the study of emotion. Finally, one could test the thesis that social organization is the result of the type of behavior present in a society. An attempted experiment along this last line is now in progress.

The use of inbred stocks of mice in bio-sociological research has some definite limitations, of course. As mammals go, mice are morphologically and physiologically very different from men. Their habits are nocturnal and subterranean rather than diurnal and open. Most important, they almost entirely lack the tendency to imitate each other which seems to be so characteristic of human beings.

Their chief usefulness arises from the control of hereditary variation and the possibility of altering it at will, and (since mice are small animals) the added possibility of strict environmental control.

Summary

1. The fighting behavior of males of several inbred stocks of house mice belonging to the Jackson Laboratory was studied, and indications of genetic differences were found between all except certain closely related strains.

2. The *C57* blacks (subline 10) and *C3H* agouti strains showed wide differences, little variability, and no overlap under the conditions tested. These, together with the *C* (Bagg) albinos are concluded to be well adapted for use in bio-sociological research.

3. The basic characteristics of the three strains appear to be:

a. *C57*—high degree of activity, tendency to make close contact with physical environment and other mice, and, under certain conditions, pacific behavior.

b. *C3H*—moderate degree of activity, tendency to avoid close contacts, easily evoked hair-fluffing reaction.

c. *C*—moderate activity and aggressiveness, no extreme behavior traits.

4. These mice are widely different from human beings in social activity but have certain special research uses because of their small size and predictable behavior.

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EMOTIONS OF MICE AND RATS

IT will be interesting to see how the studies reported above by Dr. Scott, and those of Fales and Ginsberg which Dr. Scott describes, fit into the "domestication effect" reported by Keeler and King of the Wistar Institute. (A preliminary report of the Wistar experiments was noted in this JOURNAL in October, 1941. The detailed report by Keeler and King was made at the Dallas meeting of the American Society of Zoologists.)

It is probable that the effects reported by Scott and by Fales and Ginsberg are due to multiple factors, the individual hereditary backgrounds of these strains of mice being so diverse that the effect of individual coat color genes studied by Keeler and King would not be apparent. This speculation would have to be checked experimentally. The success in battle of the initially "pacifist" *C57* black is very interesting and might even be grabbed to bolster morale in this war. And maybe rightly: the democracies don't like war and often don't do too well at it at the start. But they do seem to have a certain staying power after the show starts. But the parallel breaks down there, for democracies aren't an inbred strain of mice by any manner of means!

It will be interesting to see whether in mice the anatomical differences reported by Keeler and King in the rat bearing different alleles of the agouti series can be verified. It is even possible that rats, which are more aggressive and ingenious in their behavior than mice, have a larger variety of ways of expressing personality than mice have. These are only a few of the possibilities of research opened up by the pioneer studies which Dr. Scott reports here and that Keeler and King have reported elsewhere. The genetic study of personality traits in mammals seems to have turned a very important corner in the last few months.—ED

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"OFF THE RECORD" TWINS AND MICE

Two More Publicational "Ghosts"

THE advance of genetic knowledge on many fronts has given rise to a peculiar form of the mimeographed word. This is the bulletin or information service which is not published but circulates among a select group of specialists. It has a considerable analogy to the various "Washington Letters" which also circulate but which are not publications. It differs from them in having no subscription price. To get, you have to give of the fruits of your research labor.

The idea began several years ago with a bulletin on maize genetics issuing from the Department of Plant Breeding of Cornell University. This irregularly issued mimeographed organ contained data on maize genes and on linkage relations of those genes not yet solidly enough established to justify formal publication. Other data on maize genetics of specialized interest also circulated through this bulletin.

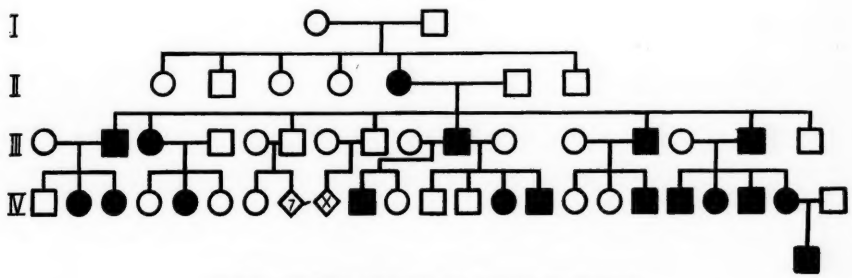
Following this came the "Drosophila Information Service" which appears increasingly in the literature as *DIS*. Since it is stated clearly on the cover of *DIS* that "this is not a publication" one sometimes wonders how so frail a paper and ink flowering can be thus canonized by formal citation. The statement on the cover reads further "unpublished material presented in this circular must not be used in publications without the specific permission of the author." Question: Does specific permission by the author mean that certain parts of *DIS* are actually publications and certain other parts are not publications? It all gets pretty complicated for an editor who is already suffering from intellectual flat feet. Is it possible that a certain amount of literary metaxenia should be allowed and that the unpublished material on each side of a permissibly published item might be just a little less unpublished than material two pages away?

A new addition to these publicationally untouchables has appeared in September,

(*Continued on page* 20)

A NEW PEDIGREE OF RECURRENT BULLOUS ERUPTION OF THE FEET

J. B. S. HALDANE AND ROSAMUNDE POOLE
Department of Biometry, University College, London, at Rothamsted Experimental Station, Harpenden, Herts



FOUR GENERATIONS OF FOOT-BLISTERS
Figure 15

III-5 and III-6 had 7 normal children of unknown sex besides an eldest daughter. III-7 and III-8 had a number of normal children. Affected individuals are shown by black symbols; squares, males; circles, females. The "diamonds" in generation four designate several persons of unknown sex.

COCKAYNE* has described two families suffering from severe blistering of the feet, occurring in hot weather only. In one case the hands also seemed to blister rather easily, but this only occurred after playing golf, whilst the blisters on the feet were very easily produced. Cockayne remarks that if these were cases of epidermolysis bullosa they were very atypical.

We here describe a new and larger pedigree of the same abnormality. The blisters are confined to the feet, and usually contain a clear fluid, though they are occasionally bloody. At their worst they are so painful as to render walking almost impossible. The severity of the symptoms does not vary greatly in different individuals. The blisters occur invariably during hot weather, but not usually during winter. However III-13 developed blisters on military service even during the coldest weather.

The pedigree of the family is given in Figure 15. I-1 and I-2 lived in Wiltshire, and both are said to have had normal feet, though the family tradition is that II-5 may have started the affection through an illness of her mother. The order of the second generation is not quite certain. II-5 was the youngest of four sisters, and older than one brother, while her first-born sister was the eldest. But the birth order of the elder brother is doubtful. There seems to be no doubt that all the other sibs in generation II were normal. We have traced several of their descendants, and found no record of the disease. II-6 was and III-15 is a dentist, and both took an intelligent interest in "the B— family curse." The junior author is an affected member of the family, and is convinced of the accuracy of the information as concerns generations III, IV, and V.

It is clear that the abnormality belongs

*COCKAYNE, E. A. "Recurrent bullous eruption of the feet." *Brit. Journ. Derm. and Syph.* 50:358-62. 1938.

to the category of "conditional" dominants, that is to say it is caused by an abnormal autosomal gene which produces a liability to blistering of the feet when in the heterozygous condition. The effect in the homozygous condition is of course unknown. But to judge from analogy with animals, and with human homozygotes for other conditional dominants, the homozygote might be very much more abnormal than the heterozygote, or even inviable.

As in Cockayne's pedigrees, the gene seems to be fully penetrant, since there is no record of its skipping a generation. If this is so, II-5 must have been a mutant. Cockayne thought it likely that his two families, both living in London, were related. However, we have been unable to trace any connection between them and that here described. Nor had Cockayne's families any known connection with Wiltshire.

It will be seen that of the children of heterozygotes and homozygous normals, 17 were affected and 11 normal. Of the 18 affected members of the pedigree, 10 are males and 8 females. In Cockayne's pedigrees, there were 15 affected and 8 normal in the sibships of which there was complete information, while 11 males and 8 females were affected. The ratio of 32 affected to 19 normal deviates markedly from equality. The difference minus one is 1.68 times its standard error. However, such a difference would occur by chance about once in 11 times, and cannot be regarded as significant. The sex-ratio is in no way abnormal, and there is no suggestion of incomplete sex-linkage.

Since three family histories of this disease have been described, and none of the 33 affected individuals could possibly be mistaken for cases of any of the known types of epidermolysis bullosa, there seems to be no doubt that we have to deal with a definite clinical entity due to a special gene, and not with the effects of one of the previously described genes for epidermolysis bullosa, modified by nature or nurture. It is of course possible that the gene concerned may be allelomorphic with the autosomal dominant responsible for many cases of this disease. Allelomorphs often produce the same effects in different areas, as in the case of the scute allelomorphs of *Drosophila melanogaster*. It is suggested that the condition be called Cockayne's disease. Since three pedigrees of it have been described within four years, it may prove to be fairly common.

Summary

A pedigree of recurrent blistering of the feet, due to an autosomal dominant gene, is described. The condition probably originated by mutation. The same or a very similar condition has previously been described in two other families by Cockayne.

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TWINS AND THE STUDY OF DELINQUENCY

THIS treatise has several distinctive features: It is the first of a series of psychiatric monographs to be published at irregular intervals by the Department of Institutions of the State of California. It represents the best collection yet captured of brief case reports on twins, of whose paired members one or both were early behavior problems, actual delinquents, or adult criminals — sometimes all three in succession. The 409 twin records were gleaned from neurological, school and child-guidance clinics and special classes throughout the United States and Canada.

Especially interesting—and Dr. Newman should take note — is the inclusion of three cases of identical twins reared apart, findings on whom have not previously been made known, at least to this reviewer. Two pairs were separated in

ROSANOFF, A. A., L. M. HANDY, and I. R. PLESSET. The Etiology of Child Behavior Difficulties, Juvenile Delinquency and Adult Criminality, with Special Reference to their Occurrence in Twins. Psychiatric Monographs, No. 1, January, 1941. State of California, Dept. of Institutions. 187 pp. $1.00 paper.

infancy, the third pair at age three years. All are now over 30 years old. I.Q.'s are available on the last-mentioned pair (72 and 89) showing a 17-point difference in favor of the girl who had had distinctly more favorable advantages. The authors do not single out this material for any special discussion, other than to mention that for all three pairs "the anti-social manifestations were not only practically identical, both in kind and amount but also synchronous in the rhythm of their occurrence."

Of 137 sets of monozygotic twins, in only 13 per cent (or in 18 cases) was one member of the pair alone delinquent or disordered. Among the 272 sets of fraternal twins (like and unlike-sex included) some 170 cases, or 63 per cent, showed only one member affected. The authors point to the "high incidence of concordant findings among dizygotic twins," (which amounts to 37 per cent of their group) and the less than complete concordance of the monozygotic group, (being 87 per cent of that group, or a relative excess of 235 per cent of the dizygotics) as a fact "which obviously discounts still further the importance of hereditary factors" and indicates causation by non-hereditary influences to which twins are jointly exposed.

The reviewer cannot refrain here from reminding the reader that even in fraternal twins, there are common hereditary factors, since siblings of any kind draw from the same parental chromosome supply, and are bound to inherit some that are alike. And that even for two structurally identical persons, there is still an element of choice and decision which can operate to determine conduct and behavior manifestations, particularly when there is little or no neurotic or psychotic involvement. This element of choice may be set down as a non-hereditary factor if you will, but it need not *ipso facto* be attributed to the environment. It is something that comes from within the individual, and as such may be influenced equally by whatever goes to shape that individual within himself. Consistency need no more be a characteristic of intra-twin mates at a given moment—howsoever identical—than of a single individual at different moments. A one-hundred per cent predictability of behavior is not a legitimate expectation for either.

A rather striking finding is the significance of sex in setting up behavior disorders, affected males exceeding affected females by 74 per cent. This in spite of the fact that offences for which girls are committed are to a large extent overlooked in boys as grounds for committment to reformatories and state institutions. Girls are sent to correctional schools because of habitual disobedience at home, or who need more protection than the home can give to safeguard them from sexual immoralities and waywardness; while boys are sent up for theft, burglary, forgery, robbery, and general incorrigibility.

A factor found to be of the highest importance in the etiology of problem behavior and delinquency is that of cerebral birth trauma. Sequals or residuals of this condition will manifest themselves for the most part in anti-social behavior in childhood, adolescence or early adult life, some 90 per cent of all the records showing an onset of difficulty before 20 years of age. To account for birth trauma, both hereditary and sex factors play a prominent part, in that both selectively account for an undue vulnerability of the brain tissues, which in turn increases the hazards of birth. In the cerebral birth trauma, inhibitory brain mechanisms are impaired or destroyed, and thus behavior is less effectively controllable or regulable in the injured individual. The authors are careful to repeat an admonition that to recognize the influence of hereditary factors in the etiology of abnormal behavior is not to claim the existence of special genetic factors determining such behavior.

As with many medically inspired researches, this study discloses a minimum of statistical analyses of the data, these being confined largely to the presentation of three tables, and the calculation of a few percentages of differential twin group behavior. But the wealth of material found in the 409 individual records,

which are presented *in toto,* will offer the more analytically trained researcher an opportunity to set up his own tabulations and analyses, and to search for clues that may throw light on problems not envisaged by the present investigators.

A point which should not be overlooked is that all the material was collected from the starting point of an affected propositus — with the other tracked down when necessary — ; that is, with an *a priori* insurance of the presence of the trait looked for in at least one member of each twin pair. It is questionable whether this kind of material — except for the cases of identical twins reared apart — has much to offer by way of illuminating the nature-nurture problem. If the trait investigated could be expressed quantitatively, and thus show variability of manifestation, in the way that intelligence does, or sense of pitch, or artistic ability or other measurable psychological characteristic, rather than as a mere presence or absence of the trait, in which a sort of "all or none" expression is recorded, then twin material could be expected to make contributions to the nature-nurture problem. But to start with affected cases, and then to try to assign to heredity or environment their fair share of causation, without varying environment, is to walk more blindly into the tangle of existing interlocking factors. Wilson and Jones have established the finding that environment for identical twins is more similar than for fraternal twins. Environmentalists can seize on this to abet their arguments. True, it is undoubtedly the existence of a common heredity which makes identicals *select* more similar environments as against the discriminations of the fraternals; the hereditarian will remember this. And the controversy goes merrily on. But nevertheless in the Rosanoff study, we cannot but be impressed with the much greater concordance in irregular behavior for identicals than for fraternals, even though fraternals do show a higher concordance than do ordinary siblings. Perhaps, some day, when a complete census of twins is available, and behavior can be measured and scaled quantitatively, we can approach twin materials via variability of manifestation and not incidence of something that after all is not an entity.

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"Off the Record" Twins and Mice

(*Continued from page* 16)

1941. This is a "Twin Bulletin." We are informed that it is the out-growth of an informal meeting which took place during the Philadelphia meeting of the A.A.A.S. in 1940 and of two memoranda subsequently circulated among approximately seventy-five individuals. It is intended as an informal news letter for those with an active interest in twin research and related subjects, for the interchange of notes, ideas, technical suggestions, queries, news, etc. It has been suggested that each issue include general discussion of some main topic.

The first issue was assembled by Dr. Morton D. Schweitzer, 411 East 69th Street, New York City. Notes to be unpublished in later issues, and inquiries regarding this new arrival in the land of the publicational ghosts should be whispered to Dr. Schweitzer.

Following the issue of Twin News comes muro-genetic wraith called "Mouse Genetic News." Number one issued in November, 1941, so we assume that it was mimeographed and distributed on Hallowe'en. "Future issues will appear if and when needed." It is the only one of the non-publications which does not claim to be such on the cover page. It does state on page eighteen that "the listing of a new mutation in Mouse Genetic News does not constitute official publication."

Mouse Genetics News goes without

(*Concluded on page* 24)

THE INHERITANCE OF "WHITE-BELLY" IN THE HOUSE MOUSE

NATHAN KALISS
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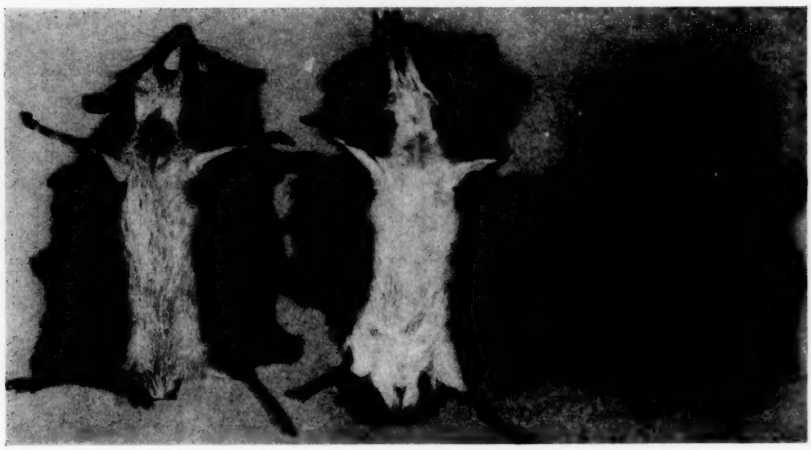

INHERITANCE OF WHITE-BELLY
Figure 16

Photograph of mouse skins showing the differences between white-belly, gray-belly, and yellow-belly. The middle skin (from animal #K 50) is an *Agouti white-belly*, and the right skin an *Aagouti vellow-belly* (#45048). The left skin is an *Agouti gray-belly* (#44730). The photograph brings out the differences in the relative amounts of yellow present in each phenotype (see text), and also shows the dark throat patch characteristic of these belly colors.

THE allelic series of coat color factors at the "agouti" locus in the house mouse is, according to Dunn[1,2], in order of dominance, *yellow* (A^Y), *agouti-light-belly* (A^L), *black-and-tan* (a^t), and *black* (a). Inbred lines of these mutants are maintained as pure breeding strains at the Columbia University laboratory. Two mutants of the series are sharply differentiated as to back and belly colors. Agouti-light-belly (henceforth designated "agouti-yellow-belly") is characterized by an agouti back and yellow belly. Black-and-tan has a black back and yellow belly. Another strain, a so-called black-and-tan-white-belly line, has been originated by selection. This strain (called Line 104) has a black back and white, or very light gray, belly.

In agouti-yellow-belly, the hairs on the back have a narrow, yellow apical band while the remainder of the shaft is black; the hairs on the belly are yellow either for most of their length, or entirely so. In "gray-belly" and "white-belly" the ventral hairs are either all white, or have a small amount of basal black pigment. The degree to which black is present determines the grayness of the pelage. Occasionally a slight amount of yellow may be present, giving the pelage a cream tint.

From a cross, made by Dr. Dunn, of an agouti-yellow-belly male (♂ #7698 of Line 101) to a black-and-tan-white-belly female (♀ #7902 of Line 104), a litter of three agouti-yellow-belly males, and an agouti-white-belly male (#9221) was obtained. A true breeding agouti-white-belly line was established by selection from crosses to male #9221. Upon Dr.

Dunn's suggestion matings were made to test the inheritance of "white-bellv" versus "yellow-belly" in relation to the agouti locus. The hypotheses to be tested were: I. is "white-belly" an allel at the agouti locus? — or II. are factors, or a factor, at other loci responsible for the belly-color segregation?

The following crosses were made (the notations for the various phenotypes are: *Ag* = agouti; *a* = black, for the colors of the back; and *yb* = yellow-belly; *wb* = white-belly; *gb* = gray-belly):

1. *Ag yb* (agouti-yellow-belly) × *Ag wb* (agouti-white-belly).
2. *Ag yb* × *a wb* (black-and-tan-white-belly, here called black-white-belly).
3. F_1 from cross (2) × *a yb* (black-and-tan, here called black-yellow-belly).
4. F_1 from cross (2) × *a wb*.
5. F_1 from cross (2) × *Ag yb*.

Crosses (1) and (2) are tests for dominance relations. Crosses (3) and (4) are segregation tests for back and belly colors.

Color classification of the progeny were made at the ages of fifteen days, one month, and four months. Final classifications were made from cured skins taken when the animals were at least four months old. This precaution was observed when it was noticed that there were marked color changes between the fifteen day pelage and the adult. Thus an animal destined to have a bright yellow belly as an adult may often appear grayish yellow at fifteen days, while a fifteen day gray-belly may be white, or creamy white, as an adult. (There is also a sex dimorphism, yellow-belly males being brighter yellow than yellow-belly females, and white-belly males being whiter than white-belly females. In both instances this seems to be due to the presence of a larger amount of black pigment in the females.)

Since the progeny of the crosses exhibited a range of belly colors from white through gray to yellow, with varying depths of gray and yellow, the final classification of the one hundred and sixteen skins recorded in Table I was made in the following manner to avoid bias of judgment: all the skins, without any knowledge as to the type of cross responsible for any of them, were spread out on a table and arbitrarily divided into three color categories; white-belly, gray-belly, and yellow-belly (see Figure 16). This classification comprised the final record for each skin.

The summarized data are presented in Table I. Portion 3b of Table I is for a cross of the F_1 from cross (2) (see above) by a blue-and-tan (blue back, due to a dilution factor, with a yellow belly). Though the numbers are small (total 116), the following conclusions may be drawn:

1. White belly (*wb*) is dominant over *yb*. The heterozygote (*wb/yb*) may be white- or gray-belly (crosses (1) and (2)). The appearance of an occasional yellow-belly may be an expression of dominance modifiers. A single factor difference appears to be involved.

2. The *wb-yb* locus is not in the agouti series of allels (crosses (3) and (4)).

If hypothesis I (see above) is correct, cross (3) should give a 1:1 segregation of *Ag gb* (or *wb*) and *a gb* (or *wb*); cross (4) should give a 1:1 segregation of *Ag gb* (or *wb*) and *a wb*. On the other hand, if hypothesis II be correct, crosses (3) and (4) should give four equivalent classes of *Ag yb*, *Ag gb* (or *wb*), *a yb*, and *a gb* (or *wb*); and *Ag wb*, *Ag gb* (or *wb*), *a wb*, and *a gb* (or *wb*) respectively.

The data clearly support hypothesis II, namely, that the *wb-yb* segregation is due to a separate locus from the agouti locus. The white-belly mutation is thus

TABLE I. Crosses of White-, Gray- and Yellow-bellied Mice.

Cross	*Black*			*Agouti*		
	wb	*gb*	*yb*	*wb*	*gb*	*yb*
1. *Ag wb* × *Ag yb*		7	2			
2. *Ag yb* × *a wb*	12	20	1			
3a. F_1 from Cross 2 × *a yb*		8	1	1	6	
3b. F_1 from Cross 2 × *blue yb*		4	19		2	5
4. F_1 from Cross 2 × *a wb*	4	1		1	4	
5. F_1 from Cross 2 × *Ag yb*		10	8			

Total animals: 116

Ag = agouti back; *a* = black back; *wb* = white-belly; *gb* = gray-belly; *yb* = yellow-belly.

a new addition to that group of genes whose specific expression is the regional differentiation of pigment formation between back and belly. Mutations of this type offer useful tools for investigating interrelations among processes of development.

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1. DUNN, L. C. A fifth allelomorph in the agouti series of the house mouse. *Proc. Nat. Acad. Sci.* 14:816-819. 1928.

2. ———— Studies of multiple allelomorphic series in the house mouse. I. Description of agouti and albino series of allelomorphs. *J. Genet.* 33:443-453. 1936.

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ARE INSECTS PEOPLE?

CLIFTON Fadiman reviews books one way; he tells how on page xliv of the prologue to *Reading I've Liked.* Robert Cook reviews books another way. In fact, several other ways. A pitcher with as many changes of pace as he has would be a big asset in any league. In the present JOURNAL for September, 1941, he starts out to review a book and ends up by giving "a listing and amplification of the reactions engendered by the book." I am glad of the precedent, because that is just what I want to do regarding the book which I have been reading on the train into New York in the mornings and on the train back to Scarsdale in the evenings, and in bed at night.*

On the day that I was nine years old, my father gave me a copy of Comstock's *Insect Life.* At the time I was an avid butterfly-chaser, pestering the merchants for empty cigar boxes, and raising caterpillars in a screen cage. I can remember the emotional reaction that the ownership of this volume engendered. Forty-one years after, Dr. Lutz' new book has called forth that reaction a second time. A book that can do that has something on the ball.

Frank E. Lutz is no stranger to the readers of this JOURNAL. To mention just one of his many publications on genetics, in 1908 he published in *The American Naturalist,* a discussion of the inheritance of clasping the hands with the right thumb up or the left thumb up. In the book under discussion, he remarks "The author of a report on some research that he has done naturally likes to have his paper quoted. It is his chief recompense for doing the work. But the notice that the publication gets is often not in proportion to the time and effort spent by the author. This thing on clasping hands took very little of my time and still less effort, and yet it has been more widely quoted than various things that cost me a great deal of both time and effort. I am not complaining; just wondering. Perhaps there is a moral, but I do not know what it is."

On his suburban lot in New Jersey, 75 by 200 feet, Dr. Lutz, working casually rather than intensively, identified

Lepidoptera	467	species
Coleoptera	259	
Diptera	258	
Hymenoptera	167	
Homoptera	75	
Heteroptera	62	
Orthoptera	29	

and so on, to a total of 26 orders and 1,402 species. By way of proving that he had not turned over his lot to the insects entirely, he mentions that in four consecutive years his place won in a newspaper-sponsored garden contest: a certificate of achievement, two bronze medals for second place, and a silver medal for first place.

How to present this material so as to interest the public, cost Dr. Lutz a great deal of thought. He discarded the taxonomic approach and the chronological arrangement, and had called the whole thing off when one evening his partner trumped his ace. "The partner being a lady who was not my wife, I could not say anything, but her husband could and did. The explanation—hers, not his—was that, when she has good cards, she

*LUTZ, FRANK E., *A Lot of Insects: Entomology in a Suburban Garden.* Pp. 304. $3. G. P. Putnam's Sons, New York. 1941.

plays them in the order that she happens to think of them. . . . Out of the 'subconscious' it suddenly occurred to me that I might try her plan with our home-lot insects."

The scientific world owes a great deal to the lady who trumped Dr. Lutz' ace. She was the instrument of releasing one of the wisest, most instructive, and most humorous books that ever came off the press, in this reviewer's opinion. The material is presented in random order, but after all, that is how the insects came to the Lutz premises; and there is an index.

Dr. Lutz tells us that *Drosophila* breeds in over-ripe fruit, not for the sake of eating the fruit itself but for the yeast that grows on such fruit. If geneticists : *Drosophila* : : *Drosophila* : yeast, there is something for geneticists in this book because of *Drosophila* alone. It is surely of scientific historic interest that while studying *Drosophila* for variations in the wing veins, Dr. Lutz found a white-eyed mutant, but having his hands full with the wing-vein problem, he gave over five descendants of the white-eyed sport to Professor T. H. Morgan. "He and his students and his students' students have been breeding descendants of those Drosophila ever since and have built up a truly magnificent structure of knowledge concerning the way in which characters are inherited. . . . This little story is no particular credit to me. If I had realized how valuable that white-eyed mutant was destined to be, I would not have been happy to give it away. However, it fell into good hands and, really, *Drosophila melanogaster* should be called Morgan's Hereditary Fly."

An outstanding feature of this informal entomology is its wise discussion of what happens when Man monkeys with the balance of Nature. Man, not liking the tent-caterpillar, cuts down the wild cherry trees, in the fatuous hope that destroying its favorite food will starve it out. The caterpillar moves over onto the apple trees, and subsequent generations, it has been shown experimentally, will stay with the apple and ignore the wild cherry. Man subsidizes his young to collect and burn the egg masses, and with the egg masses he destroys innumerable tiny wasps that parasitize not only the tent-caterpillar, but insects of economic consequence. Let the tent-caterpillar and the wild cherry alone, says Dr. Lutz, and the balance of Nature will take care of the situation better than the garden clubs can.

Important also are some pungent comments on "adaptation" and on the relative place of birds and of other insects in insect control.

Something to think about, the trend of government being what it is, is the Japanese Beetle quarantine inspector who stopped cars (when traffic was not too heavy) to inspect them for Japanese beetles, but who did not know the Japanese beetle when he saw one. Dr. Lutz deals with this situation with a charitable humor that indicates he is better adjusted to his environment and times than the reviewer, who is intensely allergic to petty bureaucrats.

I trust that my treasuring of Comstock's *Insect Life* is not out of line with my own generation. I venture that any of today's rising generation who gets the new Lutz book will prize it just as highly forty years from now. This book is really a *must* for biologists old and young.

RAMSAY SPILLMAN

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"Off the Record" Twins and Mice

(*Continued from page* 20)

charge to contributors. Others should hire a medium or dabble in E.S.P. to get the latest murine gene-gossip. Witches are definitely out as public relations council in such a connection, for no witches would be seen without her cat, and what cats do to mice is proverbial. The first issue was edited by Dr. George Snell of the Jackson Memorial Laboratory, Bar Harbor, Maine.

We greet these two new paper-ink spectres completely off the record and in a little perplexity, and wish them a long and spooky career, frightening honest-to-goodness *viva-voce* publications on dark nights.—R. C.

A NATURAL AMPHIDIPLOID FROM AN ONION SPECIES HYBRID

Allium cepa L. × *Allium fistulosum* L.

HENRY A. JONES AND ALFRED E. CLARKE*

AMPHIDIPLOIDY is of great theoretical and practical interest because of its importance in the origin of new fertile types. By a process of chromosome doubling, a shoot or a seedling of a sterile or near-sterile species hybrid suddenly becomes fertile. The classical example of the process was the spontaneous appearance of a fertile branch on a plant of *Primula kenensis,* a sterile hybrid between two species. This striking change was explained on the basis of chromosome mechanics by Newton and Pellew[10] in 1929. The amphidiploid described in this paper originated directly from the cross *Allium cepa* L. × *A. fistulosum* L. It was highly self-fertile and true-breeding in contrast to thousands of other F_1 plants from this same cross which were completely self-sterile.

Numerous crosses have been made between these two species in an attempt to combine the desirable commercial characters of both. F_1 hybrids are easily obtained, but when these have been self-pollinated only a few F_2 seeds have ever been secured. Emsweller and Jones[2] produced three F_2 seeds from a White Persian × Nebuka cross. Maeda[9] obtained a few diploid F_2 plants from the cross *A. fistulosum* var. Hidanegi × *A. cepa* var. Yellow Danvers. Levan[7] grew several F_2 plants from the cross *A. fistulosum* (strain obtained from a European botanical garden) × *A. cepa* var. Braunschweiger. All had more than the diploid complement of sixteen chromosomes, but only one was tetraploid.

Emsweller and Jones[2] secured considerable quantities of seed when the F_1 hybrid was used as the pollen parent in backcrosses to *fistulosum,* but only a very limited quantity in backcrosses to *cepa.* Levan[7] never succeeded in backcrossing the hybrid.

A cytological study of meiosis in the cross *A. cepa* var. Yellow Globe Danvers × *A. fistulosum* type Nebuka was made by Emsweller and Jones.[3] Eight bivalents were noted at first metaphase in approximately 70 per cent of the cells. Univalents in the remaining cells varied in number from two to eight. Chromatin bridges and fragments occurred frequently. Maeda[9] reported about the same frequency of bivalents as Emsweller and Jones. Levan's material was much more irregular, showing regular pairing in less than two per cent of the division figures. Levan[8] after examining the slides of Emsweller and Jones stated that the meiotic behavior in their hybrids differed very much from that found in his material. It appears reasonable to conclude that the parental forms used by Emsweller and Jones are more closely related than those of Levan, whereas those of Maeda are perhaps the most closely related of all, since he obtained some diploid F_2 plants.

Materials and Methods

For a number of years many F_1 hybrids between *A. cepa* and *A. fistulosum* have been grown in the greenhouses of the U. S. Horticultural Station at Beltsville, Md. These plants were selfed and backcrossed. Flies[6] were used in all cases in an attempt to insure thorough pollination, but no F_2 seed was ever secured with the single exception of the fertile amphidiploid. Both the F_1 and many of the F_2 amphidiploid plants were carried over winter for flowering and seed production. They were all available for

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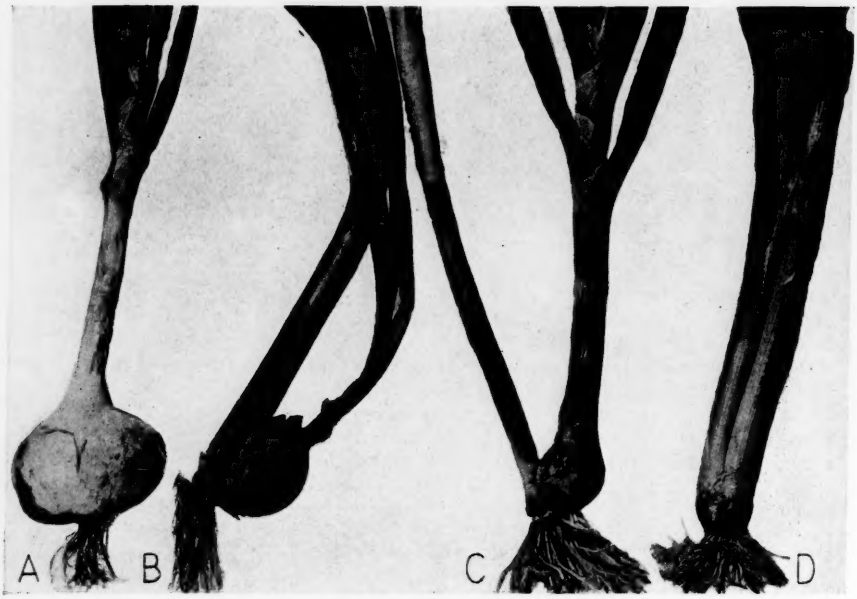


PARENTS AND HYBRIDS

Figure 17

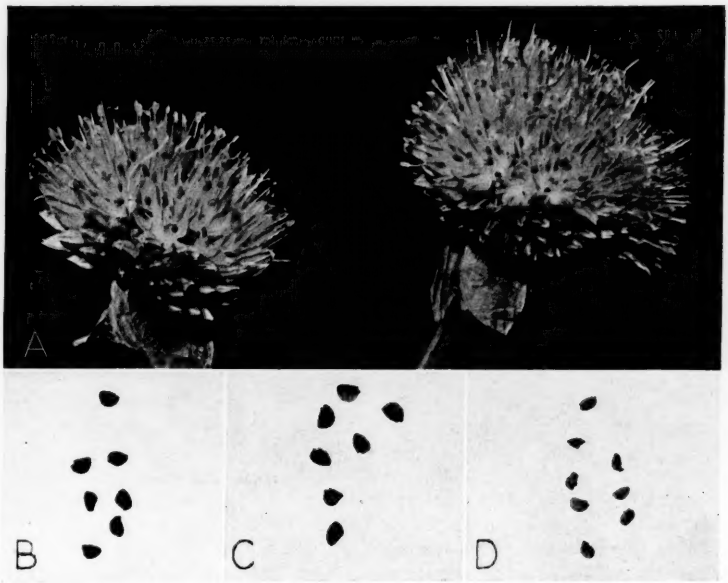
A-E—Photographs showing differences in bulbing habit between *Allium cepa, A. fistulosum,* and their hybrids. *A*—*A. cepa*; *B*—F_1 hybrid (diploid); *C*—Amphidiploid; *D*—*A. fistulosum.*

comparison with Australian Brown plants of the same strain as the female parent, and the identical Nebuka plant which was used as the male parent, and diploid hybrids of this same cross.

Size of stomata was determined by examining strips of epidermis peeled from leaves and mounted in acetocarmine. The outside length of the guard cells was measured by means of an ocular micrometer. Fifty measurements were made for each strain. Since stomatal size varies considerably on different leaves of the same plant, and even on different parts of the same leaf, care was taken to select comparable leaves and to make comparisons from as uniform material as possible.

Acetocarmine preparations of mature pollen were made to determine the amount of pollen sterility. Unstained shrunken pollen grains were obviously sterile. It was assumed that all of the large, well-filled pollen grains were viable, but no germination tests were made, and the percentage of viable pollen may therefore be somewhat lower than indicated by this microscopic examination. The size of the pollen grains was determined by mounting them in acetocarmine and measuring with an ocular micrometer.

To determine if the amphidiploid set seed after self-pollination, as readily as the parents, plants were selfed. Counts were then made of the total number of flowers per seed stalk, the number of flowers that produced seed, and the total number of seeds. The number of ovules was obtained by multiplying the number of flowers by six, since the onion produces six ovules per flower. The percentage of ovules which developed into seed gives only a rough estimate of the seed-setting capacity of the plant since there were doubtless many uncontrolled factors influencing the efficiency of the flies as pollinating agents. Care was al-



AMPHIDIPLOID FLOWERS, AND SEEDS OF PARENTS AND HYBRIDS
Figure 18

A—Inflorescence of the amphidiploid *A. cepa* × *A. fistulosum*. *B-D*—Photographs showing in size of seeds of Australian Brown (*B*); the amphidiploid (*C*); Nebuka (*D*).

ways taken to keep flies in the bags as long as the pistils remained receptive.

For the cytological studies acetocarmine smears of pollen mother cells and first microspore divisions were used. Slides were made permanent, when so desired, by mounting in euparal, as described by Whitaker.[11] Smears were also prepared by McCallum's iron-propionocarmine method.[5]

Description of Hybrid

The amphidiploid was highly self-fertile, as is shown by the fact that in 1940 it produced 1,393 seeds as the result of selfing. At all stages of growth the F_2 plants were alike in appearance and were very similar to the F_1. This failure to secure segregation, plus the unexpectedly high fertility, convinced the authors that they were dealing with an amphidiploid.

The amphidiploid shows greater vegetative vigor than either parent, as evidenced by such characters as increased height, larger stomata, larger pollen grains, greater weight of seed, and more rapid growth of the seedlings. Under similar conditions both the amphidiploid and the self-sterile diploid hybrid grow considerably taller than either of the parents.

Life Cycle. Australian Brown, like other varieties of *A. cepa,* is cultivated as a biennial. A bulb is produced the first year and seed the second. Nebuka is a perennial with only a slight tendency to enlarge in the lower sheath region. The diploid and amphidiploid hybrids are also perennial, but a much larger bulb is formed than in the Nebuka parent (Figure 17).

Leaf Characters. In Australian Brown the leaf blade is flattened on the upper surface, whereas in Nebuka it is round. The sterile hybrid exhibits this flattening on one side of the leaf, like its Australian Brown parent, but the amphidiploid has a rounder leaf, almost circular in cross section, closely resembling its *fistulosum* parent in this respect.

The stomata of the amphidiploids were significantly larger than those of either parent or of the diploid hybrid

(Table 1), the F values exceeding the 1 per cent level of significance.

Type of Inflorescence. The amphidiploid differs from both parents in the development of the inflorescence. In the two parent species and in their diploid hybrid the bract that encloses the young inflorescence splits prior to anthesis. In the amphidiploids, however, the oldest flowers frequently open while still enclosed within the bract.

In Nebuka the terminal flowers of the inflorescence are the oldest and are the first to open. They then mature progressively towards the base. In Australian Brown the opening of the flowers does not follow this regular sequence, but anthesis occurs over the whole inflorescence throughout the flowering period. The amphidiploid is intermediate between the two parents. As in *A. fistulosum,* the terminal flowers open first, being followed progressively by others toward the base of the inflorescence. It differs from *fistulosum,* however, in that additional flowers develop later over the whole inflorescence, and, as in *A. cepa,* they do not follow any regular sequence at time of anthesis, in this respect closely resembling the diploid hybrid. The type of inflorescence exhibited by the amphidiploid is illustrated in Figure 18*A*.

Flower Habit. The flowers of the amphidiploid are intermediate in growth habit between the two parents (Figure 19*B*). In Nebuka the perianth of the mature flower remains erect while in Australian Brown the perianth becomes expanded, and the flower opens more widely. In the amphidiploid, however, the perianth lobes expand more than the *fistulosum,* but not as much as the *cepa* parent.

Flower Size. The amphidiploid has larger flowers than either the Australian Brown or the Nebuka parents. The differences in size were shown by weighing fifty flowers of the tetraploid and of the two parent species. Open flowers which had not begun to shed their pollen were used (Figure 19) in five lots of ten flowers each. Pedicels were removed at the base of the perianth. The mean weights are given in Table II. The mean weight of the amphidiploid flowers is significantly greater than that of the Australian Brown which, in turn, is significantly greater than the Nebuka.

Pollen Characters. In April, 1940, the fertile F_1 amphidiploid gave a count of 29 per cent fertile pollen, and a self-sterile diploid sister plant gave 23 per cent. It was not possible, therefore, to distinguish between these two plants merely on the basis of pollen fertility, but the amphidiploid with 29 per cent fertile pollen was completely self-fertile, whereas the other F_1 plant with 23 per cent was completely sterile.

In the original amphidiploid the percentage of apparently good pollen grains from different flowers on the first inflorescence in 1941 was extremely variable, ranging from 0 to about 50 per cent. The percentages obtained in counts on different days were as follows: 1, 6, 44, 46, 13, 3, 8, 14, 16, 47, 0, 0, 49. Inflorescences which came into bloom later, however, produced much higher percentages of pollen, the actual counts being 75, 90, 72, 92, and 95.

Counts from F_2 amphidiploid plants were also extremely variable, as shown in Table III. Since the original amphidiploid commenced blooming earlier than the F_2, it was not possible to make strictly comparable counts on the same dates.

It would appear that the greater amount of pollen sterility which occurred early in the season was caused by unfavorable environmental conditions during early stages of pollen development, possibly the result of the plants being carried over winter in a cold frame and being moved into the greenhouse shortly before commencing to bloom. Later counts, ranging as high as 90 to 95 per cent, indicate that under favorable conditions the amphidipliod produced about as much fertile pollen as the parent species.

The mean length of 50 pollen grains of the two parents and of the diploid and amphidiploid hybrids is shown in Table IV. The pollen grains of Nebuka are significantly longer than those of Australian Brown. This is in agreement with the results of Levan[8] who previously pointed out that the pollen grains

of *A. fistulosum* are longer than those of *A. cepa*. The diploid hybrid produces many small, aborted pollen grains but the larger, normal-appearing grains are of the same length as those of the Australian Brown parent. The pollen of the amphidiploid is larger than that of either parent or of the diploid hybrid, the increase in length exceeding the one-per cent level of significance.

Seed Setting Capacity. A population of 318 plants secured by selfing the original amphidiploid was grown, but unfortunately many of them were lost before flowering because of damage caused by *Botrytis*. The 170 plants remaining were selfed and all set seed. A comparison was made of the seed setting of the parents, the original amphidiploid, and a few plants of the F_2 progeny. The data for the percentages of flowers that produced seed and the percentages of ovules that developed into seeds in the Australian Brown, Nebuka, and the amphidiploids are given in Table V.

These results indicate that there is an appreciable reduction in the percentage of seed produced by the selfed amphidiploids as compared with the parent species. Nevertheless, it should be pointed out that the amphidiploid produced an abundance of seed. This is in marked contrast to the diploid hybrids from the same cross, which, as previously mentioned, produced no seed after selfing.

Jones and Emsweller[6] report that plants of Australian Brown, when selfed with flies under field conditions, produce a seed set of from 12.5 to 43.5 per cent. The set of 30 and 43 per cent for the same variety reported in Table V compares very favorably with their results.

Seed Size. The mean weight of the seeds of the amphidiploid and the parent varieties was determined by weighing lots consisting of 100 seeds each. Nine 100-seed lots were weighed, three of each variety. The data are given in Table VI.

The seeds of Nebuka weighed less than those of Australian Brown, whereas the selfed seeds of the amphidiploid weighed more than those of either parent. These differences in weight are large enough to be highly significant.

The relative size of the seeds of the amphidiploid and its two parents is illustrated in Figure 18*B-D*).

Cytological Behavior

Counts at the first nuclear division of the microspore show that the amphidiploid regularly produces a haploid number of sixteen chromosomes (Figure 20).

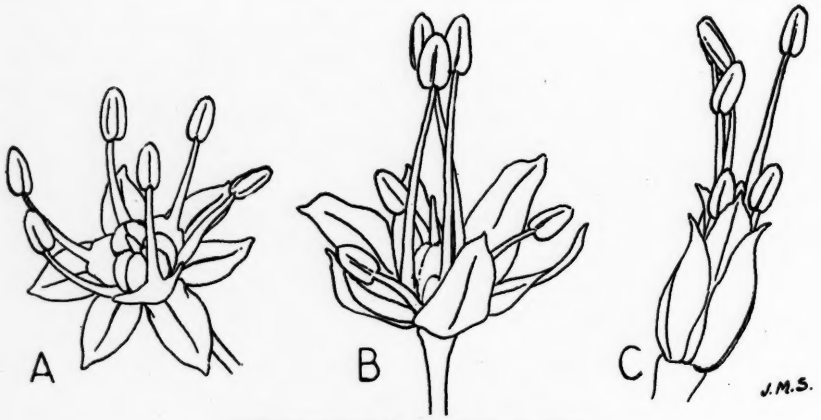
The meiotic behavior of these amphidiploids is much more regular than that found in any of the hybrids previously reported by Emsweller and Jones, Levan, or Maeda. Nevertheless, considerable irregularity has been observed.

In the early stages of the first reduction division of the pollen mother cell, for the most part, the chromosome behavior appears normal. At pachytene the pairing of the chromosomes is fairly regular. Unpaired regions have been observed in otherwise completely paired bivalents. However, such irregularities are not very common.

At first metaphase sixteen bivalents were generally found, and no quadrivalents were observed. This contrasts with Levan's tetraploid in which, although sixteen bivalents were found in the majority of the cells, univalents and quadrivalents were frequent.

The frequency of univalents in the amphidiploid and its parent species and the diploid sterile hybrid are shown in Table VII. It will be seen that the amphidiploid is much more regular in its meiotic behavior than the diploid hybrid. This diploid Australian Brown × Nebuka hybrid produced a somewhat higher percentage of univalents than the Yellow Globe Danvers × Nebuka hybrid reported by Emsweller and Jones,[3] but it is not possible to determine from the data available whether this difference is genetic in nature or merely resulted from differences in the environmental conditions under which the plants were grown.

A number of workers have shown that at first metaphase the chiasmata of the chromosome bivalents of *A. fistulosum* are interstitial and localized at the constriction region, whereas in *A. cepa* they are terminal or nearly so. The self-sterile hybrids (diploids) have chiasmata simi-



DIFFERENCES IN FLOWER HABITS

Figure 19

Left, *A. cepa* var. Australian Brown; center, amphidiploid; right, *A. fistulosum*, type Nebuka. Drawings show difference in size as well as in structure of flowers.

lar to those of *A. cepa*. In Levan's tetraploid the terminal type of chiasma formation was found. In our amphidiploid, however, both the *fistulosum*-type and *cepa*-type of chiasma formation are found in the same cell. Counts indicate that there are frequently more than eight bivalents with terminal chiasmata. No cells have been counted with more than eight bivalents with localized chiasmata.

The formation of chromatin bridges during first anaphase has been noted occasionally in the amphidiploid. In contrast fragments and chromatin bridges occurred with much higher frequency in the hybrid material analyzed by Emsweller and Jones,[4] who discussed the ways in which such irregularities may arise.

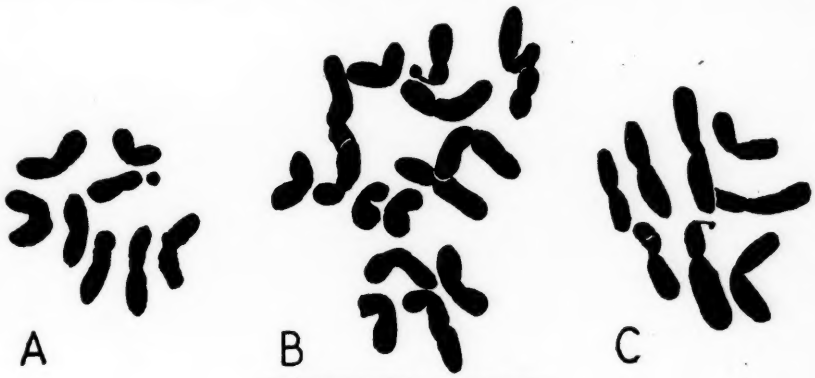
At the end of the first telophase a micronucleus is sometimes formed, but the frequency with which this irregularity occurred varied widely on different slides—from less than 1 to 7.5 per cent. A micronucleus is frequently carried into a young microspore, and several with two micronuclei have been observed.

Discussion

The amphidiploid described in this paper is true-breeding and probably originated by a doubling of the number of chromosomes of the zygote or fertilized egg. The possibility that it was produced by the fusion of two unreduced gametes appears less likely, as diploid gametes are extremely rare in the two parent species. Levan believes that his tetraploid is also an amphidiploid. It did not originate directly from the original cross, however, but from selfing the diploid hybrid, presumably through the functioning of unreduced gametes. He found that such gametes occur in the F_1 pollen of his cross with considerable frequency.

The fertility of the amphidiploid reported in this paper is in marked contrast to that of the tetraploid reported by Levan. In his tetraploid, seeds were formed with embryos but the development of these young embryos was interrupted at an early stage. The seeds atrophied and the capsules became filled with a putrefying mass until only the dry black seed coats remained. He obtained only one seed that germinated. Our amphidiploid, on the other hand, produced large, normal seeds abundantly.

The regular occurrence in the same cell of some bivalents with random and others with localized chiasmata is markedly different from the behavior found in the sterile hybrids, and in Levan's tetraploid in which only the random type of chiasma formation was found. How-



CHROMOSOMES COMPARED

Figure 20

Camera lucida drawings of chromosomes at first microspore division. *A*—Australian Brown (n=8); *B*—Amphidiploid (n=16); *C*—Nebuka (n=8). ×-1420.

ever, Maeda[9] reported that some of his F_2 and backcrossed plants produced both random and localized bivalents in the same cell, the random type being the more frequent. Emsweller and Jones[1] studied seventeen plants backcrossed to *fistulosum,* ten of which produced random and several localized chiasmata. They sometimes found both random and localized bivalents in the same cell, although all plants appeared to be predominantly of one type or the other. However, the occurrence of both types of chiasma formation in the same cell of the amphidiploid reported in this paper does not agree with Emsweller and Jones' suggestion that the type of pairing is controlled by a single pair of genes.

Levan[8] recently put forward the view that "The irregularities of the chiasma formation in F_2 and in backcrosses of the *cepa* × *fistulosum* cross are due to the occurrence of recombination types with unbalanced chiasma conditions, types which under normal conditions would soon be exterminated. Thus, each bivalent of an individual has no special tendency to assume the one type or the other, but owing to an unbalanced genotype in this respect a lack of coordination during the chiasma formation causes strictly localized chiasmata to be formed at the same time and in the same nucleus as random chiasmata." He points out, however, that Maeda's results are not consistent with this theory since Maeda found that a particular bivalent always showed the same type of chiasma formation.

It might be suggested that when in the amphidiploid, two chromosomes of the *cepa* genom pair at first metaphase the chiasmata are randomized. When two chromosomes of the *fistulosum* genom pair the chiasmata are localized. When a *cepa* chromosome pairs with a *fistulosum,* as in the diploid hybrid, the bivalent is of the random type. According to this theory if the sixteen chromosomes derived from the *cepa* parent always paired among themselves, and the sixteen *fistulosum* chromosomes did likewise, then at first metaphase the hybrid would produce eight bivalents with localized and eight with terminal chiasmata. The excess of bivalents with terminal chiasmata found in the amphidiploid reported in this paper might be attributed to some pairing of *cepa* with *fistulosum* chromosomes. This view, however, will not explain the behavior of Levan's tetraploid. It is hoped that a more detailed cytological study will throw additional light on this problem.

Summary

A fertile amphidiploid has been obtained from a cross between *Allium cepa* var. Australian Brown and *A. fistulosum* type Nebuka. Second generation plants are very uniform in appearance and set seed readily.

This amphidiploid shows greater vegetative vigor than either parent, as evidenced by increased height, larger stomata, larger flowers, larger pollen grains, greater weight of seed, and more rapid growth of seedlings. It is a perennial like Nebuka.

The haploid number of chromosomes in the amphidiploid is sixteen. Meiotic behavior is fairly regular. Sixteen bivalents are generally found at first metaphase. Fragments, chromatin bridges, and micronuclei were observed but were not as common as in the diploid hybrid. During meiosis both the random and localized type of chiasma formation are found in different bivalents in the same cell.

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TABLE I. Size of stomata in Nebuka and Australian Brown onion varieties and their hybrids.

Variety	Length of guard cells*	S. E.
	Microns	
Australian Brown	39.4	± 0.43
Nebuka	41.9	± 0.16
F_1 diploid	41.8	± 0.61
Amphidiploid	51.7	± 0.45

* Means of 50 measurements.

TABLE II. Mean weight in milligrams of ten flowers of the Nebuka and Australian Brown onion varieties and their tetraploid hybrid, based on weights of 5 samples of 10 flowers.

Variety	Mean weight of 10 flowers	S. E.
	Mgms.	
Australian Brown	367.6	± 15.89
Nebuka	278.4	± 8.24
Amphidiploid	497.6	± 42.12

TABLE III. Percentage of fertile pollen produced by F_2 amphidiploids of Australian Brown x Nebuka.

	Date of Count		
	March 9	March 19	March 26
Plant No.	Percent	Percent	Percent
37	85	93	73
38	--	8	8
42	--	1	59
49	--	1	82
52	--	--	84
54	--	--	53
56	74	40	78
57	--	91	85
58	--	40	43
60	--	--	80
63	--	84	--
64	--	9	--
70	--	--	73
73	--	--	70

TABLE IV. Size of pollen grains in Nebuka and Australian Brown onion varieties and their hybrids.

Variety	Length of pollen grain	S. E.
	Microns	
Australian Brown	36.0	± 0.31
Nebuka	39.6	± 0.32
F_1 diploid	35.7	± 0.32
F_1 amphidiploid	46.7	± 0.66

TABLE V. Percentage of flowers that produced seed and percentage of ovules that developed into seeds in Australian Brown, Nebuka, and the amphidiploids.

Variety	Plant No.	Number flowers*	Number flowers with seeds	%	No. ovules	No. seeds	%
Australian Brown	4634	132	119	90	792	336	42
	4636	164	148	90	984	297	30
Nebuka	487	146	124	85	876	452	52
	494	225	109	48	1350	450	32
Amphidiploid	F_1-15	672	158	23	4056	211	5
	F_1-16	861	356	63	3372	658	17
	F_1-19	445	333	75	2670	563	21
	F_2-26	392	179	46	2352	268	11
	F_2-64	437	187	43	2622	392	15
	F_2-182	261	153	59	1566	253	16

*Each count based on one inflorescence.

TABLE VI. Mean weight of seeds of Nebuka and Australian Brown onion varieties and their tetraploid hybrid, based on weights of 9 samples of 100 seed.

Variety	Mean weight per 100 seeds *
	Milligrams
Nebuka	190.2
Australian Brown	491.9
Amphidiploid	574.9

* Difference required for significance to 1-percent point: 13.68 mgs.

TABLE VII. Chromosome pairing in Nebuka and Australian Brown varieties of onion and their hybrids.

Type of pairing	Frequency			
	Nebuka	Australian Brown	F_1 diploid	F_1 amphidiploid
16_{II} + 0_I	-	-	-	92
15_{II} + 2_I	-	-	-	5
8_{II} + 0_I	107	100	64	-
7_{II} + 2_I	1	4	51	-
6_{II} + 4_I	0	0	20	-
5_{II} + 6_I	0	0	5	-
4_{II} + 8_I	0	0	2	-

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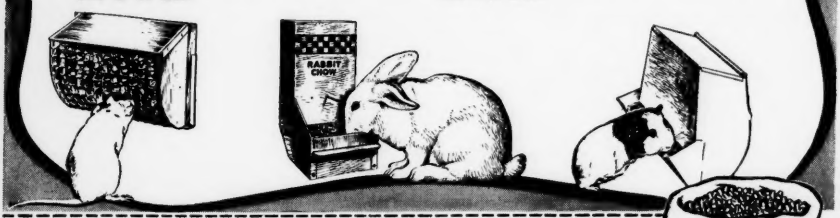

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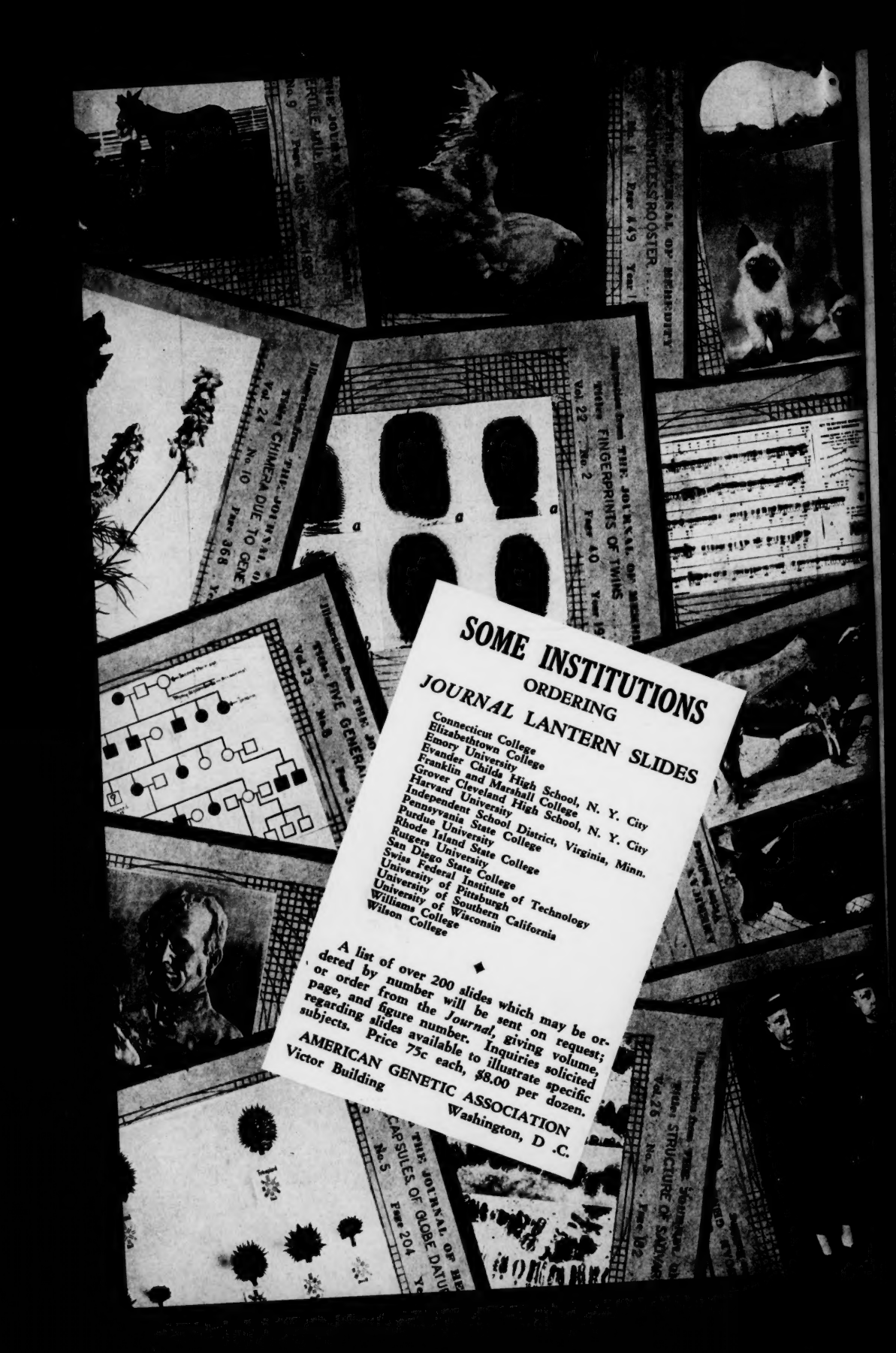

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